

Developing Minimally Impactful Protocols for DNA Analysis of Museum Collection Bone Artifacts

by

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Abstract

This study has addressed the issue of destructive testing on museum collection artifacts from two perspectives. Firstly, interviews were conducted with museum professionals from across Canada to identify their specific concerns regarding access to their collections. Secondly, this information was then used to help develop a minimally impactful DNA sampling technique that may lead to greater access to museum collections for research.

The development of this sampling technique involved successive rounds of testing conducted on bone samples including modern samples, unmodified archaeological samples, and museum artifacts from two different museums. The DNA sampling was done using a precision hand drill which produced a small amount of bone powder collected for analysis and species identification.

The results from the study indicate that it was possible to develop a successful, comprehensive and reliable minimally impactful DNA sampling technique that is tailor-made to address the concerns and ethical responsibilities of museum professionals.

Keywords: Ancient DNA; Bone Sampling; Minimally Destructive; X-ray Imaging; Species Identification; Museum Collections

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List of Acronyms

aDNA	Ancient DNA
BCE	Before Common Era
BLAST	Basic Local Alignment Search Tool
bp	Base pair
BP	Before present
BSA	Bovine serum albumin
CE	Common Era
ddH ₂ O	Double-distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
mtDNA	Mitochondrial DNA
PCR	Polymerase chain reaction
rpm	Revolutions per minute
SDS	Sodium dodecyl sulphate
SFU	Simon Fraser University
UBC	University of British Columbia
UV	Ultraviolet

Glossary

Accessioning	The process of creating a permanent record of an object, assemblage, or lot received from a source for which a museum has custody of, by assigning a unique control number to said object.
Ancient DNA	The DNA found in ancient remains. Ancient DNA is typically characterized by low quality and quantity.
Amplicon	A fragment of DNA that is produced by PCR amplification. In this project specific sequence fragments were targeted through the use of target-specific primers.
Amplification	An artificial increase in the number of copies of an authentic aDNA fragment into millions of copies through the PCR process.
Anneal	The second step of the PCR process; recombining single-stranded DNA into double-stranded DNA following separation by heat.
Autolysis	The destruction of cells or tissues by their own enzymes, especially those released by lysosomes.
Conservation	The action of prolonging the existence of significant objects by stabilizing and maintaining its existing condition, and minimizing any further deterioration and/or damage.
Contamination	The presence of DNA from a source foreign to the sample being analyzed. These contaminants can be co-extracted alongside, or instead of, the target DNA and mask the amplification of the target DNA.
Denature	The first step of the PCR process; double-stranded DNA separates into single-stranded DNA with the application of heat.
Depurination	A chemical reaction caused by hydrolysis, which results in the removal of a purine (adenine or guanine) base from DNA.
Diagenesis	A complex of changes caused by chemical, biological, and physical processes acting on a dead specimen over time.
DNA polymerase	An enzyme involved in DNA replication. It helps catalyze the polymerization of deoxyribonucleotides into a DNA strand.
Extend	The third step of the PCR process; added nucleotides attach to the primer and elongate in order to create a new single-strand of DNA.

Gel electrophoresis	A technique that results in the size separation of charged molecules. Electricity is run through an agarose gel causing the DNA to migrate in the direction of the current. Shorter fragments migrate faster than larger fragments, and this creates a “band” which can be visualized with dye.
Hydrolysis	Water molecules form new chemical bonds with parts of the DNA molecule, which result in breaking down and damaging the DNA.
Inhibitor/Inhibition	Any factor that prevents the amplification of DNA during the PCR process. Inhibitors are typically co-extracted alongside DNA and generally exert their effects through direct interaction with DNA or by interfering with DNA polymerases.
Mitochondrial DNA	Circular DNA molecule found in mitochondria, inherited only through the female line. Mutates faster than nuclear DNA and is found in greater quantities in a cell.
Oxidation	The process by which water-derived hydroxyl or superoxide radicals modify bases or distort the helix of the DNA strands.
Polymerase chain reaction	A molecular technique used to amplify DNA exponentially. PCR relies on thermal cycling to denature, anneal and extend DNA templates during amplification.
Primer	Short synthetic DNA sequences that serve as a starting point for DNA synthesis. Primers are used to target specific sequences of DNA during PCR amplification through complementary binding to template DNA.
Restoration	The actions taken to modify the existing material and structure of an object in order to return it to a known earlier condition (eg, as new).

Chapter 1.

Introduction

1.1. Research Objectives

The physical products of archaeological excavations are often collected and exhibited in museums around the world, which function as repositories for these culturally significant materials as well as places of learning and exploration for the public. As a result of museums' roles as archaeological repositories they are privileged in having a unique longitudinal perspective regarding human and natural history and represent an unparalleled resource which could be utilized in an astounding array of research questions (Graves and Braun 1992; Mundy *et al.* 1997; Suarez and Tsutsui 2004).

The genesis for this research project was the assumption that museum professionals are hesitant to allow destructive testing done on their collections as this conflicts with their ethical responsibility to conserve and act as stewards of the archaeological material under their care (Adriaens 2005; Baker 1994; Barker 2010; Bolnick *et al.* 2012; Lehmann *et al.* 2005; Mundy *et al.* 1997; Rohland *et al.* 2004; Scarre and Scarre 2006; Thomsen *et al.* 2009; Wisely *et al.* 2003). If a minimally destructive or impactful method could be developed that strikes an appropriate balance between the various concerns of museum professionals, it might lead to greater access to museum collection artifacts for research purposes and more open and collaborative partnerships between researchers and museum professionals.

The goals of this research project are twofold. The first was to conduct a series of interviews with a range of museum professionals from three areas of Canada to discuss their collections and to identify any concerns they have regarding destructive testing.

The second was to develop and apply a minimally impactful sampling technique for extracting aDNA from bone artifacts, with the specific purpose of applying this technique to bone artifacts curated in museum collections. Minimally impactful is being defined here as a technique that consumes or damages a small portion of a sample without creating any unexpected internal or external damage, leaves the sample available to re-analysis, and does not affect its long term preservation or stability.

The museum interviews conducted generated a wide array of viewpoints on several topics including museum policies, potential and involvement in research, and destructive testing on museum artifacts. This data was used to examine our initial assumption regarding access to museum collections for research purposes and to design a technique in response to the concerns raised by museum professionals.

Developing and optimizing this minimally destructive technique involved multiple rounds of testing aimed to assess its effect on bones of different preservation conditions. A morphological preservation scale was used to formalize the selection criteria for this study and x-ray imaging was used to assess both the pre and post-sampling structural integrity of the bones and identify any subsequent damage created by the technique. The technique itself involves using a precision micro-drill to drill into the sample at controlled speeds to extract a small amount of bone powder. It is the bone powder that is then consumed in the DNA extraction process leaving the remainder of the sample intact. The goal of the DNA analysis is to provide authentic, reliable species identifications for all the samples tested from a small amount of bone powder.

This study is the first of its kind to directly identify the concerns of museum professionals by conducting a series of interviews, and develop a tailored sampling technique to address their specific. Hopefully a minimally impactful technique for DNA sampling that suits the needs and concerns of museum professionals will increase access to museum collection artifacts for research and in turn strengthen collaborations between researchers and museums.

1.2. Thesis Organization

The remainder of this thesis is organized into six chapters, which are further divided into appropriate subsections.

Chapter 2 provides the context for this research. The history and development of the aDNA discipline is included along with a review of the factors that damage DNA and need to be subsequently overcome when attempting aDNA sequencing. This chapter also includes a review of the relevant aDNA extraction methods and protocols relevant to the development of a minimally destructive technique. This chapter concludes with a look at the history of aDNA analysis and museum collections, and the added challenges that exist when undertaking this kind of work.

Chapter 3 presents the results of the museum interviews. This chapter covers the selection criteria, participant selection, and the analysis of the interview data. It concludes with a discussion that ties together the main themes and conclusions drawn from the interview data.

Chapter 4 outlines the materials and methods used in the development of this minimally invasive technique. First is a review of the sample material and their archaeological contexts, followed by information on the sample preparation methods, drilling techniques and DNA analysis protocols used.

Chapter 5 presents the results of the DNA analysis. The morphological preservation scale used as part of the sample selection criteria is discussed, followed by the results of the morphological and DNA based species identifications, and the results from the pre and post-analysis x-ray imaging comparison. This chapter concludes with a summary of the results.

Chapter 6 discusses the results of this study in more detail, tying them together with the aims of this project. Firstly the minimally invasive drilling method is discussed including a breakdown of its major strengths and challenges. This is followed by the technique's implications to museums and museum collections, and the chapter concludes with a selected list of areas for future research.

Chapter 7 is the conclusion which outlines the final thoughts and themes discussed throughout this thesis and its overall significance.

Chapter 2.

Background

2.1. Ancient DNA Analysis in Archaeology

The first DNA extracted from an ancient specimen occurred in 1984. Higuchi *et al.* (1984) successfully extracted DNA from dried muscle tissue from a quagga (*Equus quagga*), a zebra-like species that became extinct in 1883. What followed was a flurry of research on ancient specimens in an effort to advance the newly born ancient DNA (aDNA) research discipline. In these early years, soft tissue was the main source of aDNA (Baker 1994; Doran *et al.* 1986; Higuchi *et al.* 1984; Mundy *et al.* 1997; Pääbo 1985; Pääbo 1989; Pääbo *et al.* 1988; Thomas *et al.* 1990; Thomas *et al.* 1989).

Hagelberg *et al.*'s (1989) DNA extraction from human bone from specimens 300 to 5,500 years old proved that it was possible to extract aDNA from sources other than mummified soft tissue. In the decades since, bone has become the standard material for aDNA recovery. This is as a result of bone being the most common type of biological material excavated from archaeological sites, as its material and structure is one of the best for resisting degradation (Brown and Brown 2011; Campos *et al.* 2012; Hagelberg and Clegg 1991; Lassen *et al.* 1994). Research has also indicated that aDNA extracted from bone contains fewer PCR inhibitors than aDNA extracted from other materials such as sediment or mummified soft tissue DNA, and so it is more easily amplified (Lassen *et al.* 1994; Rodrigues 2012). Thus, this research has developed a drilling technique specifically for use on ancient bone material. Although many techniques have developed in the last 30 years, and will continue to improve, there are still many practical challenges that exist when conducting aDNA research which are detailed below.

2.1.1. Post-Mortem Damage to Ancient DNA

The primary challenge that faces aDNA researchers is the reality of molecular degradation that occurs during post-mortem decay, also called diagenesis (Lyman 1994; O'Rourke *et al.* 2000). While an organism is living, the integrity of their DNA is maintained by enzymes which repair damaged DNA. However, once an organism dies these enzymes no longer function and the body, and the DNA within that body, begin to degrade and self-destruct. This process is called autolysis (Brown and Brown 2011; Gilbert 2006; Gilbert *et al.* 2003; Herrmann and Hummel 1994; Matisoo-Smith and Horsburgh 2012).

These processes can be further specified into damage that directly affects DNA preservation; hydrolysis and oxidation. Hydrolysis is the breakdown of the N-glycosyl bonds between the sugar and the base in the presence of water. Oxidation is the process by which water-derived hydroxyl or superoxide radicals modify bases or distort the helix of the DNA strands (Hösset *al.* 1996; Lindahl 1993; Lyman 1994; O'Rourke *et al.* 2000; Poinar 2003; Wandeler *et al.* 2003). There is some research that suggests that histone proteins incorporated into nuclear DNA can offer some protections from damage, however their absence in mtDNA renders it very susceptible to a range of biochemical attacks (Gilbert 2006). In addition to these internal chemical damage processes, natural external phenomena such as bacteria, fungi, insects, soil acidity and temperature further accelerate the degradation process (Herrmann and Hummel 1994; Matisoo-Smith and Horsburgh 2012).

As ancient remains are subject to these processes over vast periods of time their cumulative effects produce very highly fragmented aDNA strands. Although there are many factors that could increase or decrease the effect of these processes, the age of the ancient remains is an important factor in the DNA's quality. The fragment length of these strands rarely exceed 500 bp, which has clearly limited the researchers ability to amplify longer sequences for analysis (O'Rourke *et al.* 2000; Pääbo 1989; Willerslev and Cooper 2005). DNA molecules are one of the least stable molecules within an organism's cells. These cells are very sensitive to hydrolysis and oxidation and will rapidly deteriorate soon after death fragmenting the DNA. Apoptosis, or cell death, results in the efficient and rapid cleavage of DNA into small fragments. One of the most

important factors in long term DNA preservation is the rate at which cellular enzymes, called nucleases, can be stopped (Poinar 2002).

2.1.2. Environmental Conditions

Although these chemical post-mortem processes occur in all living cells, one of the most important factors that archaeologists and museum professionals must contend with is the state of the depositional environment in which the remains were buried, and the storage conditions in which they are kept post excavation (Burger *et al.* 1999). Based on the chemical rate of decay immediately after death and neutral external environmental factors (such as soil acidity and temperature) it has been estimated that 100,000 years is the time beyond which DNA will be degraded beyond retrieval (Gilbert 2006), although as of 2013 this date has been significantly pushed back to 400,000 with the retrieval of a cave bear aDNA sequence (Dabney *et al.* 2013; Hofreiter *et al.* 2014). This timespan is greatly reduced if unfavourable conditions are present, such as highly acidic soil, a highly oxidized environment or high temperatures. Furthermore, the impact of an environment does not end with the primary depositional environment. Secondary storage environments can cause further degradation from human handling, elevated temperatures and greater access to oxygen (Campos *et al.* 2012; Phillips and Simon 1995; Wandeler *et al.* 2003).

One of the most agreed upon factors in extending long term DNA preservation is the use of cold temperatures, both naturally in primary depositional environments and after excavation in storage facilities (Burger *et al.* 1999; Campos *et al.* 2012; Smith *et al.* 2003; Willerslev and Cooper 2005). Higher temperatures increase the depurination of DNA, while colder temperatures halt or slow down the biochemical breakdowns that occur in the body and simultaneously preserve the body from parasites and bacterial attack (Hofreiter *et al.* 2014; Höss *et al.* 1996; Lindahl 1993; Willerslev and Cooper 2005).

Other factors that favour long term aDNA survivability include neutral to slightly alkaline soils, rather than acidic environments; arid environments as opposed to water rich environments which would speed up the rate of hydrolysis; and oxygen deprived

environments which slow down the process of oxidation and well as providing a low-bacterial environment (Burger *et al.* 1999; Poinar 2002). It is thought that with completely ideal conditions, aDNA might be able to survive up to one million years (Willerslev and Cooper 2005).

2.1.3. Contamination and Ancient DNA Authentication

In the decade after the first aDNA publications it looked like aDNA could be extracted from anything including some inorganic materials, up to millions of years old and answer many archaeological, or indeed paleontological, questions (Gilbert 2006). Examples include the extraction of Cretaceous Period dinosaur DNA and insect DNA from amber (Cano *et al.* 1993; Woodward *et al.* 1994). However, investigations into these studies demonstrated that the results were either irreproducible or contaminated (Austin *et al.* 1997; Gilbert 2006; Sidow *et al.* 1991; Zischler *et al.* 1995). This demonstrated that contamination of aDNA with modern DNA is a serious problem for researchers, and that the question of how to authenticate aDNA results must be resolved.

To this end, researchers acknowledge the need for purposeful contamination controls in all aDNA research and have adopted relatively standard contamination controls for the aDNA extraction process (Cooper and Poinar 2000; Handt *et al.* 1994; Kemp and Smith 2005; Poinar 2003; Yang and Watt 2005). The area where contamination controls have not been universally adopted is in the original archaeological fieldwork, although this has been suggested (Yang and Watt 2005). These widely accepted contamination controls include: a physically isolated aDNA laboratory (including a separation between aDNA lab space, PCR and modern DNA lab space), negative, blank and positive extraction and PCR controls, reproducibility, independent replication, and physical precautions (including masks, gloves, body suits) to reduce modern DNA transference (Cooper and Poinar 2000; Handt *et al.* 1994; Kemp and Smith 2005; O'Rourke *et al.* 2000; Poinar 2003; Yang and Watt 2005). Additional contamination controls that vary between labs include decontamination with bleach (both the samples and the lab tools and surfaces), the use of UV irradiation to destroy contaminants (both in the samples and in the lab), and the use of blind testing during

DNA extraction (Cooper and Poinar 2000; Handt *et al.* 1994; O'Rourke *et al.* 2000; Yang and Watt 2005).

These contamination controls have multiple purposes during the research process. The first is that they aim to remove contaminants that already exist in the sample, such as the use of bleach and UV irradiation on the samples. The second is that controls such as the use of masks, gloves, and protective clothing with prevent any further contamination of the samples while they are in the lab. And the third is to authenticate the sequences produced from the lab by using negative, blank, and positive controls throughout the process and by repeat amplifications. With the use of these combined contamination controls it is possible to produce accurate and reliable aDNA analyses (Cooper and Poinar 2000; Handt *et al.* 1994; O'Rourke *et al.* 2000; Yang and Watt 2005; Yang *et al.* 2005).

2.2. Ancient DNA Extraction Methods and Protocols

After 30 years of exploration, development, and refinement there are many aDNA sampling techniques and extraction methods available from which to choose that overcome many of the natural, environmental, and technical challenges detailed above. These methods also offer a variety of choice to researchers, archaeologists, curators and collections managers, and interest groups such as descent communities. Depending on the factors that are valued by each invested party, such as maximizing the chances for success by removing a larger amount of bone, or being as least destructive as possible, or keeping the specimen's outward appearance the same, different techniques may balance those interests better than others.

Within the literature these sampling methods can be loosely grouped into two main categories; destructive sampling methods, and minimally destructive or non-destructive methods. This division has been created for the purposes of this discussion and is based upon the language this is used in the publications themselves (the issue of lack of standardized language within the literature is addressed later in this chapter). The following sections summarize the main research and techniques within each method category.

2.2.1. Destructive Methods

As outlined above, one of the paramount concerns to aDNA researchers is the risk of contamination. Ancient remains are at risk of contamination from any number of sources including their depositional environment, the circumstances under which they were excavated, handling from the excavation team, or lab contamination (Yang and Watt 2005). As a result, steps must be taken throughout the extraction process to reduce the risk of contamination (Kemp and Smith 2005; O'Rourke *et al.* 1996; O'Rourke *et al.* 2000; Yang and Watt 2005).

The sample used for the extraction may be an entire object if it is small, such as a fish vertebra or tooth, or a piece may be removed from a larger object, such as a long bone. This extraction sample will be completely consumed to attain the desired aDNA sequence.

The first step of many aDNA extraction processes is the decontamination of the sample's surface. This can be accomplished by either physically removing the surface of the sample and/or chemically decontaminating the sample. Physical removal of the surface can be performed with a scalpel, a rotary drill with a decontaminated abrasive bit, or sandpaper (O'Rourke *et al.* 1996; O'Rourke *et al.* 2000). Chemical decontamination can be accomplished by bathing the sample in bleach (5% sodium hypochlorite, NaOCl) which will destroy the contaminant DNA by cleaving the DNA strands and breaking them down into smaller pieces, and eventually individual bases (Kemp and Smith 2005). A bath of hydrochloric acid (HCl) can also be used in conjunction with this step. The use of ultraviolet (UV) light can also be used throughout the extraction process to further destroy contaminants on the samples as well as to destroy possible DNA residues on the extraction equipment and facilities (Hummel and Hermann 1994; Kemp and Smith 2005; Yang and Watt 2005). Although decontamination is a vital part of the aDNA extraction process it is important to bear in mind that aggressive or prolonged use of these decontamination techniques will also increase the chances of damaging the already degraded aDNA that might exist in the samples (Rohland and Hofreiter 2007; Yang and Watt 2005).

Once the initial decontamination has taken place the actual aDNA extraction can occur. The first step of the process is to physically reduce the sample, or subsection of the sample, to powder. This can be accomplished by grinding the sample with any kind of tool (coffee grinder, hammer, mortar and pestle, etc.) as long as they are sterile (O'Rourke *et al.* 2000). By homogenizing the sample and increasing its surface area, the reagents and enzymes in the lysis buffer used to release the DNA contained in the cells can work more effectively (Hummel and Hermann 1994; O'Rourke *et al.* 1996). This lysis buffer contains ethylene diaminetetra-acetic acid (EDTA), sodium dodecyl sulfate (SDS) and proteinase K, and it is used to break down the matrix of the bone and release the DNA into the lysis solution (Hagelberg and Clegg 1991). The EDTA breaks up the cell walls, decalcifies the bone and stabilizes the DNA, the SDS produces an isotonic milieu to stabilize free nucleic acids and the proteinase K digests the proteins that bind the DNA. This solution is then incubated overnight in a state of constant agitation (Hummel and Hermann 1994; O'Rourke *et al.* 1996; O'Rourke *et al.* 2000; Yang *et al.* 1998).

The next step in the extraction process is to separate the DNA from the cellular debris in the buffer. There are four main methods for accomplishing this: phenol, silica, boiling and chloroform (Hummel 2003). The most widely used are the phenol and silica methods. The phenol-chloroform extraction method initially breaks down the crystalline minerals, proteins and complex lipids in the sample. The next phase separates the nucleic acid fraction from the other components by repeated separation into hydrophobic and aqueous phases. Finally, the solution is passed through a membrane filter which concentrated the DNA extracted from the sample (O'Rourke *et al.* 2000; Barnett and Larson 2012; Yang *et al.* 1998). In this method, the chloroform is used to remove the traces of phenol from the solution, as phenol is a strong PCR inhibitor (Hagelberg and Clegg 1991; Hummel and Hermann 1994). A drawback to this method lies in the fact that any inhibitors present in the DNA extraction that can filter through the membrane will also be concentrated alongside the DNA (O'Rourke *et al.* 2000; Yang *et al.* 1998).

The silica method uses silica particles which have a high binding capacity for DNA molecules and uses that capacity to isolate the DNA (Höss and Pääbo 1993; Yang *et al.* 1998). This method extracts DNA in a high concentration of guanidinium thiocyanate (GuSCN) which has the ability to lyse proteins and facilitates the binding of

DNA to the silica particles (Höss and Pääbo 1993; O'Rourke *et al.* 2000). This solution is incubated and then pelleted by centrifugation (O'Rourke *et al.* 2000; Yang *et al.* 1998). After centrifugation, the silica pellet is washed in a modified GuSCN extraction buffer, with ethanol and with acetone (O'Rourke *et al.* 2000). The DNA is eluted from the silica with a TE buffer (Yang *et al.* 1998). These purification steps are vital as silica particles are powerful PCR inhibitors, and so every effort must be made to ensure that the final extract is free of residual silica particles (Höss and Pääbo 1993; O'Rourke *et al.* 2000; Yang *et al.* 1998). In the literature, the silica methods seems to be favoured, likely attributed to a higher amplification success rates (although lower DNA yields) and fewer PCR inhibitors (O'Rourke *et al.* 2000; Rohland and Hofreiter 2007a). However, these conclusions have been challenged as being unsystematically tested although the techniques remain effective and popular (Rohland and Hofreiter 2007b).

2.2.2. Non-destructive and Minimally Destructive Ancient DNA Analysis

Non-destructive extraction methods represent a common, and very well established option within the available extraction techniques which best address issues of visual destruction and re-analysis. It is important to note that although the term “non-destructive” is used freely in this section - as this is the term that is used in the published literature itself - this paper asserts that the only absolutely non-destructive technique is one that does not test the object directly (but rather tests the associated material such as soil). Ancient DNA extraction is, by definition, a destructive process as it is necessary, at minimum, to break open the cellular structure of the sample in order to release the DNA for analysis. In this sense, the major non-destructive techniques summarized in this chapter are still destructive or impactful on a cellular level (the effects of which have not been documented over long intervals of time), but do have the benefit of leaving the object looking undamaged and available for further DNA analysis.

The destructive nature of virtually all forms of DNA analysis can present a problem to those interested in accessing the information that can be gained from studying these remains and artifacts, but are also charged with their long-term preservation and safekeeping, such as archaeologists and museum professionals

(Adriaens 2005; Baker 1994; Bolnick *et al.* 2012; Mundy *et al.* 1997; Rohland *et al.* 2004; Thomsen *et al.* 2009; Wisely *et al.* 2003). This problem is further exacerbated with small specimens, such as teeth, seeds or hair, where even limited sampling may destroy the entire sample or important morphological features (Phillips and Simon 1995; Thomsen *et al.* 2009; Wisely *et al.* 2004).

Research addressing this issue in the aDNA field roughly divides into two main bodies of work: minimally destructive sampling and non-destructive sampling. However, there is no standardization of terminology within these studies and often the terms “minimally destructive”, “non-destructive”, “non-invasive” and “consumptive” are used interchangeably when describing similar methods. For example, Pichler *et al.* (2001) and Cobb (2002) describe a “non-destructive” drilling technique that extracts DNA from the root cavity of teeth, while Shiroma *et al.* (2004) describe a “minimally destructive” technique that extracts DNA from the pulpal cavity of teeth. These publications approach the sampling with drills, and stress the ability to restore the appearance of the tooth with wax or a polymer filler, however they define the destructive impact of their technique very differently. These distinctions are in fact very important to those in the conservation field (Adriaens 2005) and having a more standardized language by which to refer to testing methods would advance the cause of researchers wishing to gain access to museum collections.

Techniques described as minimally destructive, tend to focus on sampling in unobtrusive locations (Horváth *et al.* 2005; Wisely *et al.* 2004), with an emphasis on minimizing the impact to the external appearance of the sample (Shiroma *et al.* 2004; Wisely *et al.* 2004). For example, one of the most well researched examples of minimally destructive sampling is conducted on teeth, which are highly resistant to environmental degradation due to the strong nature of the tooth enamel and are an excellent source of DNA (Hummel and Hermann 1994; O'Rourke *et al.* 2000; Shiroma *et al.* 2004). Shiroma *et al.* (2004) used a drilling method to cleanly separate the crown of the tooth, extract the pulpal and dentinal tissue from the inside of the tooth, and then reattach the crown of the tooth with wax so that it appears undamaged. These sampling procedures are still acknowledged as being destructive, however, if the sample as a whole is not structurally

or morphologically compromised for long term preservation or other forms of analysis, the impact can be considered minimal (Wisely *et al.* 2004).

The research on non-destructive sampling is more complicated, as there are a variety of techniques that fall into this category. Some of these “non-destructive” techniques involve drilling, filing, or removing portions of a specimen, and are the same in function and focus as the “minimally destructive” methods described above (Cobb 2002; Mundy *et al.* 1997; Pichler *et al.* 2001). The truest non-destructive technique involves extracting DNA from the material surrounding the remains, such as permafrost or soil. However this technique is limited by the availability of accompanying soil/permafrost contexts, as well as being limited to more recent specimens due to DNA preservation levels (Thomsen *et al.* 2009).

By far the most common and consistent non-destructive technique involves a digestive submersion method. The sample is soaked in a GuSCN buffer (or equivalent) which will release the DNA into the buffer to be later purified using silica binding (Bolnick *et al.* 2012; Gilbert *et al.* 2007; Phillips and Simon 1995; Rohland *et al.* 2004). It is the buffer that is used for the DNA extraction thus this technique does not involve any macro physical destruction of the sample. After the sample is soaked in the buffer for approximately 15 minutes, it is thoroughly rinsed which halts further chemical digestion, allowing it to be returned to the museum collections. However, the possibility of chemical alterations to the sample not visible to the eye cannot be excluded (Bolnick *et al.* 2012; Rohland *et al.* 2004).

During a recent conversation with Dr. Deborah Bolnick, lead author of one of the key publications in this area of research (Bolnick *et al.* 2012), she reported that some of the first few teeth that were tested using the non-destructive soak protocol experienced additional visually observable tissue loss from the tooth root sometime after the study ended. Because other teeth from the initial study did not show any additional tissue loss, and the many teeth processed with the non-destructive soak protocol since 2012 have shown no additional tissue loss with time, Bolnick suggested that these initial samples may not have been rinsed sufficiently with water after soaking, perhaps allowing the buffer to continue digesting the tissue. Bolnick also reported that there is some variability

in how teeth respond to this technique, possibly linked to tooth fragility (as noted in the original 2012 paper), geographic location, soil environment, or museum storage conditions. The digestive buffer sometimes leads to tissue loss in the tooth root and/or cracking along the edges of the crown. When discussing the application of this technique to museum artifacts she said that curators and collections managers should know that the method seems visibly non-destructive for many teeth, but not all of them uniformly. She suggests a trial run on one or two teeth from a site or collection to assess the technique's impact, and if there is no tissue loss then the technique could be applied to a larger sample. If this soaking technique does adversely affect the samples, Bolnick suggested that a different protocol, such as the one proposed in this thesis, might provide a better alternative for those remaining samples. Future studies assessing the long-term impact of these techniques would be an immense benefit to researchers, and would assure museum professionals of this technique's reliability for use on museum collections.

Given the lack of standardized terminology evident in these publications, this study will adopt an adaptation of the definitions proposed by Adriaens (2005):

Non-invasive:	Sampling which does not require the removal of any part of the object.
Micro-destructive:	Sampling which consumes or damages a few picoliters of the object.
Minimally-destructive/ impactful:	Sampling which consumes or damages up to a few milligrams of the object.
Non-destructive:	Sampling which will leave the complete object available for re-analysis, as well as other types of analysis.

Having consistent and precise terminology is an important step when consulting with curators, collection managers, and conservators regarding requests to access their collections.

2.3. Ancient DNA Analysis and Museum Collections

Museums are not purely places of education and display, they are an invaluable component of and contributor to multi-disciplinary research and contemporary social concerns. Museums all over the world house vast biological and cultural collections which have been used to study pathogens, vectors of disease, environmental contaminants, global climate research, global agriculture, extinct species and habitat conservation, human evolution and migration, population genetics, and more (Baker 1994; Suarez and Tsutsui 2004; Wandeler *et al.* 2007). The contributions that museums and their collections have made to a variety of disciplines are impressive, but the importance of museums to the birth and development of aDNA studies cannot be overstated.

As stated at the beginning of this chapter, the first successful aDNA extraction was conducted on 140-year-old quagga (*Equus quagga*) tissue, stored in the Museum of Natural History, Mainz, West Germany (Higuchi *et al.* 1984). The field of aDNA studies took massive technological leaps in the following decade, and the majority of these foundational studies were conducted on museum collection specimens, some up to 13,000 years old (Doran *et al.* 1986; Hagelberg *et al.* 1989; Higuchi *et al.* 1984; Pääbo 1985; Pääbo 1989; Pääbo *et al.* 1988; Thomas *et al.* 1989, 1990). These studies proved to be the beginning of decades of aDNA research in archaeology and have revolutionized the available research techniques the way carbon 14 dating did decades previously.

Museum collections continue to be an important resource for archaeologists, though they present researchers with a number of specific technical challenges in addition to the ones detailed earlier in this chapter. For example, as a secondary storage environment, museums environments can be responsible for further DNA damage and degradation (Burger *et al.* 1999; Campos *et al.* 2012; Phillips and Simon 1995; Wandeler *et al.* 2003; Willerslev and Cooper 2005). This degradation could result from human handling by collections managers or conservators, elevated or fluctuating temperatures and humidity, chemicals from conservation efforts, greater access to oxygen (example, if the artifacts are stored on open shelving instead of sealed box storage) and being stored

in acidic paper (Herrmann and Hummel 1994; Lindahl 1993; Matisoo-Smith and Horsburgh 2012).

There is evidence to suggest that there is a rapid decrease in the amount of amplifiable DNA from an object within the first 30 years of storage in a museum (Rohland *et al.* 2004; Wandeler *et al.* 2003). Some of this degradation might be attributed to the object's loss of equilibrium with its burial environment, which will cause further breakdown and destabilization (Rodgers 2004). However, any unfavourable storage, handling, or conservation conditions will also greatly reduce the timespan that good quality DNA will be retrievable (Campos *et al.* 2012; Phillips and Simon 1995; Wandeler *et al.* 2003). These factors will need to be considered by museum professionals as well as researchers whenever DNA research is being proposed so that appropriate and viable samples can be selected, if they are open to this kind of testing at all¹.

The following chapter details the results of several museum interviews that were conducted for this study. Although the rest of the information gained from these interviews is explored in the next chapter, two questions asked of these museum professionals are relevant to the issue of storage and preservation. The interview participants were asked about the record keeping at their institution through the years, as well as the storage conditions for their bone collections. All of the participants noted that the record keeping, including conservation and treatment work, recording storage locations, accessioning information and intake documents, prior to the 1990's were limited and spotty. Usually, only the current location and storage conditions of artifacts are recorded in any detail, and so valuable information that could be extremely helpful in selecting viable artifacts for research is lost when previous records detailing the storage history of the objects are overwritten. Digital databases have changed everything regarding museum documentation practices, however older institutions such as the Royal Ontario Museum or Museum of Vancouver which are over a hundred years old,

¹A list of recommended best practices for museum professionals looking to undergo aDNA research can be found in Appendix D.

still have ongoing projects to reconcile older forms of documentation with new digital systems.

Likewise, current storage locations and conditions for collections are well recorded, but the complete history of every location and condition an artifact has been exposed to is non-existent. The storage conditions themselves also vary from museum to museum. Some museums have their collections stored on site in a variety of controlled conditions (some described as macro and micro-environments), while others may take advantage of off-site dedicated storage facilities. One such example is the Museum of Ontario Archaeology which stores some of their collections at the Sustainable Archaeology (<http://sustainablearchaeology.org/>) facility in London, Ontario which is a dedicated archaeology storage repository. The realities of these common museum record limitations, combined with the realities of DNA degradation in museum environments means that it may be difficult to identify ideal samples for DNA extraction. There are many variables within museums that affect the chances for a successful DNA extraction, unfortunately for researchers these variables are very difficult to account for and control due to the limited and inadequate nature of collection records and data.

2.4. Background Summary

The very destructive nature of the majority of DNA sampling techniques may present a barrier for curators, collection managers and conservators (Adriaens 2005; Baker 1994; Bolnick *et al.* 2012; Lehmann *et al.* 2005; Mundy *et al.* 1997; Rohland *et al.* 2004; Thomsen *et al.* 2009; Wisely *et al.* 2003). For the last 30 years the very ethical codes that archaeologists and museums have strived to abide by have stressed stewardship, conservation and preservation as key factors in their professional behaviour (Green 1984; Lynott 1997; Lynott and Wylie 1995; McGill 2014; Scarre and Scarre 2006; Zimmerman *et al.* 2003). As the focus of this research is to provide a minimally destructive option for DNA sampling with the hope of increasing access to museum collections, a number of interviews were conducted with museum professionals in order to assess the impact that the destructive nature of DNA testing has on their decision making process. The results of these interviews are detailed in the following chapter.

Chapter 3.

Museum Interviews

3.1. Museum Selection

Instead of targeting specific museum professionals to interview, the museums themselves were selected first in order to capture as much institutional diversity as possible. Primary sampling was determined by museum size. There are many metrics by which a museum's size could be determined (i.e., collection size, visitor count, ticket profits, research funding, community impact etc.). For this study museum size was based on the number of full time employees (not including janitorial/maintenance staff, volunteers, or guides). This decision was made largely out of convenience as employee numbers are easy to find online and confirm, and are a discreet number that is easy to compare between the institutions. In contrast, something like "collection size" is harder to establish and confirm, and indeed the definition of what constitutes a collection could differ between institutions which would make it hard to compare between them.

For the purposes of this study, museums were classified as "small", "medium" or "large" institutions. Small museums are institutions where there are 1-10 employees, medium museums have 11-30 employees, and large museums have 31+ employees. Ten interviews were conducted for this study: four interviews from large museums (40%), two from medium museums (20%), and four from small museums (40%) (see Table 1 for a summary). In the museum selection process there was an attempt to capture some geographic diversity across Canada as well. Six of the interviews were conducted on museum professionals from British Columbia museums (60%), three from Ontario museums (30%), and one from the Northwest Territories (10%).

3.2. Participant Selection and Recruitment

Before any potential participants were contacted permission had to be granted by the SFU's Office of Research Ethics board. The study was approved (2016s0040) and potential participants were mainly contacted by e-mail. A total of ten people agreed to be interviewed, and the interviews were conducted from March to July 2016. Most of the interviews were conducted in person, while one interview was done over Skype and one other was done over the phone.

A key factor in selecting a potential interview participant was their intimate contact and involvement with their museum's collections, so curators (50% of participants) and collection managers (10% of participants) were the preferred candidates, although a variety of museum positions are represented among those who agreed to be interviewed (see Table 1 for full position descriptions). Among the responsibilities of these interview participants were collections management and safety, artifact acquisitions and registration, exhibitions, conservation, policy writing, programming, volunteer management and research. The participants' employment at their respective institutions ranged from a year in their current position to an impressive 44 years of continuous service and institutional knowledge.

Table 1: Summary of interviews conducted.

Museum	Museum Abbreviation	Museum Size	Participant	Position Title	Interview Date
Royal Ontario Museum	ROM	Large	Chen Shen	Senior Curator, VP of World Cultures	April 7, 2016
Royal British Columbia Museum	RBCM	Large	Grant Keddie	Curator of Archaeology	March 18, 2016
Prince of Wales Northern Heritage Centre	PWNHC	Large	Sarah Carr-Locke	Director	April 1, 2016
Canadian Museum of Civilization ²	CMC	Large	George F. Macdonald	CEO, retired	March 10, 2016
Museum of Vancouver	MOV	Medium	Wendy Nichols	Curator of Collections	April 5, 2016
Vancouver Maritime Museum	VMM	Medium	Duncan MacLeod	Collections and Curatorial	March 30, 2016
Gulf of Georgia Cannery	GGC	Small	Heidi Rampfl	Collections Manager	July 12, 2016
Museum of Ontario Archaeology	MOA	Small	Nicole Aszalos	Museum Curator	March 16, 2016
Museum of Archaeology and Ethnology	MAE	Small	Barbara Winter	Director	March 11, 2016
Anonymous	-	Small	Anonymous	Anonymous	March 17, 2016

² Since this participant's employment at this museum, the Canadian Museum of Civilization was renamed the Canadian Museum of History.

3.3. Interview Structure and Analysis

Before any interviews took place each participant was asked to sign a Consent Form³ which provided all the study details pertaining to the interviews. There was an option offered in the Consent Form to remain anonymous which one participant requested. As seen in Table 1 and throughout this chapter, this person is referred to as “Anonymous”. As a courtesy, a draft of this chapter was also sent to the interview participants to review and approve of how their comments were used in the study.

The interviews were semi-structured (Bernard 2006; Hesse-Biber and Leavy 2011; Palys 1992) and generally followed the same progression of questions⁴. This interview structure ensured that each interview participant would be asked similar questions, and so their answers would be comparable. However, it left room for organic, comfortable conversation to develop, which could take the interview in a unique direction. Interviews lasted anywhere from 30 to 120 minutes. The interview questions were loosely grouped around two themes: DNA and bone related questions, and collection management related questions.

The majority of the interview participants gave permission for audio-recording, so data collection was based on both written notes from the interviews as well as transcripts from the audio-recordings. During the analysis of the data, it was noted that some questions were not asked of every participant. Thus follow up questions were sent by e-mail as necessary.

The qualitative data generated from these interviews were manually analyzed for patterned and divergent answers. Sample size and comparably structured interview guides meant that no qualitative data analysis software was needed to aid in the analysis.

³ A copy of the Consent Form can be found in Appendix A.

⁴ A copy of the Interview Questions can be found in Appendix B.

3.4. Interview Results

The results detailed in this section are the responses to the DNA and bone related questions from the first part of the interview. The collection management related questions from the second part of the interview were discussed in the previous chapter.

Eight out of the ten people interviewed (80%) indicated that their institution had bone artifacts, or artifacts with bone components, as part of their collections. The two respondents (Gulf of Georgia Cannery [GGC] and Anonymous) who indicated that their museums did not collect bone artifacts worked at small museums with very specific collections, and both in regional focus and in temporal time, and so did not have the range of collections that could be found in some of the other museum collection. For example, the GGC, a National Historic Site of Canada, was built in 1894 and closed in 1979 and the collections (the cannery building itself, assembly line machinery, archive material) represent a very specific time period.

Of the museums that do have bone artifacts, the approximated numbers vary: the Vancouver Maritime Museum (VMM) approximated under a thousand bone artifacts; the Prince of Wales Northern Heritage Centre (PWNHC), Museum of Vancouver(MOV), and Museum of Archaeology and Ethnology (MAE) approximated bone artifacts in the thousands; the Canadian Museum of Civilization (CMC), Royal British Museum (RBCM) and Museum of Ontario Archaeology (MOA) approximated tens of thousands of bone artifacts, and the Royal Ontario Museum (ROM) approximated hundreds of thousands of bone artifacts in their collections. What was more difficult to account for when comparing the interview data was what was considered an “artifact”, and what was considered an “archaeological collection”. Largely depending on the size of the museum, there could be different departments that differentiate their collections in different ways (natural history, ethnology, archaeology etc). These differences in collection classifications may result in an over or under-estimation of collection size. It also resulted in a variety of answers when the interview participants were asked the date ranges for their bone collections; material ranged from modern ethnographic bone pieces to natural history fossils millions of years old.

The eight respondents that indicated that their museums did have bone artifacts in their collections were asked if, to their knowledge, any DNA research had been conducted on any of their archaeological bone artifacts. Five of the eight respondents (62.5%) indicated that no DNA work had ever been done on their archaeological collections to their knowledge. However, Dr. Chen Shen (ROM) and Mr. Grant Keddie (RBCM) noted that their museums had biodiversity or natural history departments that are separate from the archaeological departments. They stated that DNA work had been done on the natural history collections, but not on archaeological remains. Dr. George F. Macdonald (CMC) and Dr. Barbara Winter (MAE), representing 25% of respondents, indicated that there has been DNA work conducted on their collections. Dr. Macdonald indicated that the CMC had done a great deal of cooperative DNA analysis on human remains as part of ongoing land claims and other negotiations with the Provincial and Federal Governments as well as research in to early North Pacific Coast populations. Dr. Barbara Winter allowed museum artifacts to be used as research samples in SFU Department of Archaeology student research projects. The final respondents (12.5%), Dr. Sarah Carr-Locke was unsure of whether or not DNA work had been done on their bone collections.

Some follow up questions were asked of some of the respondents based on their answers and anecdotes to the previous question. Mr. Keddie and Dr. Winter, were asked about the information that was gleaned from the DNA testing on their material. Mr. Keddie stated that the information that was gained by DNA testing the natural history collections was absolutely worth the destruction. Mr. Keddie added that he strongly encourages all kinds of testing on museum collections, and that he believes museums should be collecting and curating with future testing in mind.

Although Dr. Winter stated she also believes in the importance of museum collections being used for research (“They’re curated for a reason”), she shared a different perspective regarding destructive testing. Dr. Winter recounted her experience of loaning a collection to a researcher for testing. The PWNHC (where Dr. Winter was previously employed) had a collection of roughly 34 bird eggs collected in the 1920-30s. A researcher from the University of Alberta’s Department of Biology wanted to test the eggs as part of a research project on the effects of DDT. It was specified in the loan

agreement that the testing was to be completely non-destructive. However, when the researcher was contacted about returning the collection it was revealed that the entire egg collection was pulverized and consumed in the analysis. The museum was not even sent a copy of the research results which were the product of their now completely destroyed collection.

Dr. Winter stated this experience made her less welcoming to the idea of destructive testing. She also stated that in her experience there is an issue with researchers using museums as a resource, without the courtesy of sharing their findings with the museum or asking any research questions that are of interest to the museum. She feels without a fair, responsible collaboration between researchers and the museums that provide their samples there is little benefit to the museum, and the research potential is often not worth the destruction done to the collection artifacts.

A follow up question inquired about what kinds of questions a researcher might ask that would also be of interest to each museum. Dr. Winter noted that she felt that it was absolutely important to use the collections to contribute to the knowledge of the world, but curators and collections managers don't necessarily know how to apply DNA techniques to those collections to ask meaningful questions. This is why she feels a partnership between researchers and museum professionals is so vital for successful research projects. Mr. Keddie stressed that there are endless questions that could be asked of museum collections as they capture a depth of time seen nowhere else. He used animal remains as a way to study climate change over time as an example. Mr. Duncan MacLeod (VMM) answered this question very practically and pointed out that DNA analysis on special artifacts would be very useful for museum professionals as loan agreements, conservation work, and insurance contracts require detailed information regarding the material each artifact is composed of. The more detailed these records can be the better for the museum. Mr. MacLeod stated that whether it be for exhibitions, online database records, or loan agreements with other institutions, more accurate, correct information on the collections is important.

A key question when discussing destructive testing in museums is whether or not these institutions have a destructive testing policy or protocol. Nine interview participants

answered this question, with only one museum that had a dedicated archaeology specific destructive testing policy. Ms. Nicole Aszalos (MOA) who participated in these interviews was the curator that wrote the policy, which focuses on providing a justification of the scientific value of performing the testing, the rarity of the object being requested, the amount of damage likely to occur from testing and the proposed location of the damage, and the necessity to share the results of the research with MOA after completion. Three museums, the RBCM, PWNHC and CMC (33% of respondents) have destructive testing policies but are written with biodiversity or natural history departments in mind. These policies can be adapted by archaeology departments on a case by case basis, however the overall concerns detailed in the policies can differ from the concerns associated with archaeological collections.

The respondents for four museums, the MOV, VMM, GGC and MAE (44% of respondents), indicated that they have no destructive policy on the books. If and when destructive analysis requests are submitted, the curators and collections managers proceed on a case by case basis. And Dr. Shen (11% of respondents) indicated that there is a “best practices” protocol established at the ROM, which now advocates for following the directions of the individual collection curators. Any requests for destructive testing get passed on to the appropriate collection curator who will make a judgment in consultation with the appropriate conservators. Although this protocol is more formal than the 44% previously mentioned, it also boils down to a case by case decision.

With this information in mind, the interview participants were asked if any other kind of destructive testing has ever been conducted on their respective archaeological collections. Of nine respondents, seven museums, the ROM, RBCM, PWNHC, CMC, MOV, MOA, and MAE (78% of respondents), said that other forms of destructive testing had been conducted on archaeological collections. These testing methods include radiocarbon dating, thin sectioning and imbedding, stable isotope analysis, and copper sourcing. Some respondents also indicated that they considered x-ray and CT imaging to be a kind of destructive test, or they have collaborated with those that have felt this form of analysis includes an element of destruction. Ms. Heidi Rampfl (11% of respondents) indicated that no destructive testing had been done at the GGC, and Mr.

MacLeod (11% of respondents) said that he was not sure what testing had been done over the years at the VMM.

Of the seven respondents that indicated that other forms of destructive testing had been conducted, they indicated that these analytical techniques provided useful information and that the experiences were largely positive. Ms. Wendy Nichols shared that the MOV had radiocarbon dating and stable isotope analysis conducted on two human remains in their collections as part of an ongoing repatriation program. The remains in question had no provenance or associated records, and so the resulting dates and dietary information were used in tandem as a tool to identify, or in this case to exclude, descendant communities in an effort to respectfully return the remains.

When asked early in the interview what they felt the greatest barrier to DNA testing on museum collections is, eight of the ten interview respondents (80%) answered that the destructive nature of the test and the damage to the artifact was the main barrier. This was by far the greatest consensus among those interviewed. Other answers included lack of knowledge on the part of the museum professionals (20%), respect and need for permission from the source/ancestral community (20%), a lack of encouraging policy (10%), financial cost (10%), the lack of benefit to the museum (10%), and the uniqueness/rarity of the artifact (10%).

When asked if there were some artifacts that the participants would allow or not allow destructive testing on, nine out of the ten respondents (90%) answered yes. Many interview participants indicated that there are some artifacts that might be considered too valuable/unique to have any destructive analysis performed on them, while some other artifacts would inspire less hesitation. Only Mr. Keddie (RBCM) felt that all artifacts should be available for analysis (unless the source community is against the research), especially rare and unique artifacts as those are the objects that we want to learn the most about. He stated, "Given new techniques that do minimal damage I don't really see anything that we wouldn't allow to be studied."

As 90% of respondents stated there would be artifacts they would not allow destructive testing on, they were asked what key factors would weigh in that decision. The most common answer with eight out of ten respondents (80%) in agreement was

the uniqueness/rarity of the object in question. It was repeatedly stated by multiple interview participants that if the museum had a collection of a hundred similar objects there would be little worry in allowing testing on a subset of that collection. Whereas a request to perform destructive testing on a “completely unique” object would be cause for more consideration.

Other consistent “key factors” from the participants included the research objectives or value of the contribution gained from the analysis (70%), the destructive nature of the testing/ long term stability of the object (70%), concern for the “displayability”/aesthetic value of the object (60%), and respect for the source community and the necessity for gaining permission in certain circumstances (50%). More unique opinions regarding “key factors” that came from the interviews included exploring alternative, non-destructive methods before approving a destructive testing method (20%), the importance of preparing for the future considering what techniques may be available and leaving material for the future (10%), and the provenance or significance of the object in question (10%).

When asked an open ended question about the future of DNA analysis and museums, seven out of the nine respondents (78%) indicated that the strength of DNA analysis techniques partnered with museums is in creating more complete records of the past and increasing our knowledge through research. Seven out of the nine respondents (78%) also indicated that they felt there was potential for further expansion between researchers looking to do DNA analysis and museums. Dr. Winter summarized it best by saying, “I think the potential is huge! Museums are data banks waiting to be mined, spanning history, biology and geology. We can find the answers to questions we don’t even know we have yet. The potential is only limited by our own imaginations and creativity.”

3.5. Discussion

Although the destructive nature of DNA analysis is by no means the only barrier to applying this technique to museum artifacts, it was by far the most consistent answer given by the ten interview participants. These interviews indicated that the rarity and

uniqueness of the object itself is a key factor in the thought process behind allowing or disallowing destructive testing, as well as the potential intellectual contribution the analysis may provide. The overwhelming consensus from the interview participants was that when considering destructive testing a balance needs to be struck between the museums' responsibility to conserve and preserve their collections, and their responsibility to utilize those collections to make a valuable contribution to society through increased knowledge and information.

Many of the participants asked, why do we have these collections if not to use them? One interview participant, who wished to remain anonymous, captured this feeling very eloquently:

I do think there should be a balance between collections being collected for the purpose of preservation - that they must remain intact and preserved fully - I think there should be some of that. But there should also be some - we need to learn more about these things. So there has to be that balance.

The same sentiment was also echoed by Dr. Carr-Locke (PWNHC):

Yes we are looking outward to see what else is out there, but I think we also need to spend the same amount of time looking inward and see how much do we know about what we already have...It is our ethical responsibility to make sure that we are using this material that we have to learn as much as we can and to form different relationships with communities.

These quotes underline the importance of striking a balance between conservation and research, and fully utilizing the collections and resources already available. Dr. Carr-Locke's comment also draws attention to the possibilities of what can be done with the resulting research products. Public engagement and relationship building between museums and different communities was a consistent theme during these interviews. Many respondents stated that research can be used as a means to keep museum collections relevant. Dr. Shen (ROM) stated:

The 21st century museums are no longer treasure hordes. They're here to bring the collections alive to engage the public. To allow our public – especially young children and students - to be more engaged in the museum by making the past relevant to the present.

Mr. Keddie added that research is essential for museums, as museums have a responsibility to the public to stay current and provide updated information. He stated, “The public expects us to have good quality, factual information. It’s a place they can trust.”

It is gratifying to know that none of the museum professionals interviewed for this project were outright against destructive testing, however they all stated that it is important that any destructive testing be purposeful and the information gleaned from the research be worth the risk. Destructive testing of any kind is a risk, however if the right balance is struck between the research questions, the artifacts, and the testing methods then it can be a risk worth taking.

Chapter 4.

Materials and Methods

4.1. Sample Material





Given the willingness of museum professionals to allow destructive testing on collection artifacts and objects where important research gains can be expected, it is incumbent upon DNA researchers to use destructive techniques that are as minimally impactful as possible. To this end, this thesis further aims to develop, test, and optimize a minimally impactful drilling technique which could provide the very balance described during the above interviews. This technique was applied to several rounds of test material. The selection of these materials aimed to target varying degrees of bone preservation as well as a cross-section of bone depositional contexts.

4.1.1. Morphological Preservation Scale

In order to optimize this technique and have it be considered reliable, it needed to be tested on a variety of different materials representing a range of preservation levels, and a range of bone sizes and thicknesses. In order to achieve testing through this range, the test material for this study was selected based on an amalgamated version of previously published bone gross preservation scales (Haynes et al. 2002; Petchey and Higham 2000; Stafford et al. 1988). This scale categorizes bone into five classes ranging from very well preserved bone to highly degraded bone, based on observable features such as luster, porosity, cracking and density (Table 2). Class V, the most degraded class of bone on this scale, has been eliminated from this study as it is generally too unstable to drill into (crumbles at physical touch). Any archaeological or museum artifact's preservation that could be classified as Class V would be ill-suited for this technique. However, if it is possible to attain the same information from Class V

material as it would be from a better preserved piece suitable for exhibition (for example, a question regarding the general makeup of the collection, versus a question regarding a certain artifact intended for display), and if museum professionals are willing to sacrifice these highly degraded pieces in their collections to destructive testing, it may not be necessary to impact any of the better preserved artifacts. If however, it is necessary to sample artifacts better suited to display this technique offers a minimally impactful technique for sampling Class I to IV material.

Table 2: Morphological preservation scale based on similar scales (Haynes et al. 2002, Petchey and Higham 2000, and Stafford et al. 1988) and select examples of archaeological test materials.

Class I	Class II	Class III	Class IV	Class V
High compressive and tensile strength; spiral and conchoidal fracturing; waxy luster; dense bone matrix	Bone is chalky with loss of conchoidal fracturing; exterior still hard and waxy	Interior and exterior of bone are chalky; surface hardness decreases and porosity increases	Continued decrease in hardness and increase in porosity	Soft, easily pulverized bone if no inorganic replacement occurs, low density; hard if inorganic replacement has occurred
				Excluded

By testing this drilling method on a variety of bone preservation conditions it will become evident if and when the drilling threatens the structural integrity of the bone, and to what degree. It will also establish which states of preservation are most suitable for this kind of sampling. This information is absolutely essential for creating a minimally destructive method reliable enough to be used on museum artifacts.

To reduce the variables present in these rounds of testing, the majority of the sample material was limited to land mammals thereby avoiding any discrepancies in comparing the drilling method and the subsequent results (for example, comparing drilling larger, compact mammal bones to small, thin fish bones). Making comparisons between land mammals will keep the technical observations more consistent. The sample bones were largely blindly selected, however morphological identification confirmed that the majority of the selected samples were land mammals and fit the testing criteria.

4.1.2. Test Round 1: Modern Test Samples

Several modern samples of pig and turkey bones were selected for initial drilling in order to assess the power of the drill and identify any preliminary issues (see Table 3). As this was modern material it was all from Class I and very well preserved. This round of testing also allowed for the exploration of different drilling setups. For example, a large desktop vice was experimented with to see if immobilizing the sample would be an effective option. In fact, the vice was too aggressive for the samples and posed additional risks of cracking if pressed too tightly. Experimenting with the vice also highlighted the need for a greater freedom of movement for effective drilling, and for maximizing bone powder collection.

Table 3: Preliminary drilling issues anticipated during the first round of testing.

Anticipated Drilling Issues	Addressed	Not Addressed
<i>Drill Setup:</i>		
Sample immobilization with a vice	✓	
Drilling site visibility issues (experimentation with a mirror to see under the sample)	✓	
Bone powder collection methods	✓	
<i>Drill Technique:</i>		
Kickback from the drill (possibly related to speed)	✓	
Drill speeds and a correlated increase in heat produced as a result of increasing the drilling speeds	✓	
Measuring the difference in heat production at different drill speeds		✓
Measuring the pressure exerted on the drill while drilling, which could cause more friction and increase heat produced		✓
Amount of powder produced (cortical vs trabecular bone)	✓	
Ease of drilling with different bit sizes	✓	
Size and depth of the holes created during drilling	✓	

This round of testing was designed to assess the drill, identify potential issues, and experiment with possible drilling setups and was done without the need for DNA analysis. The most effective drilling setup discovered in this process was used for the remainder of the testing and is detailed below in the “Drill Technique” subchapter.

4.1.3. Test Round 2: Modern Degraded Test Samples

Thirty unidentified samples were randomly selected from the animal remains excavated from a buried context in Port Coquitlam, British Columbia which are housed in the SFU Centre for Forensic Research. The material was excavated in 2002-2003, and the animal material recovered from the site is comprised of modern degraded bones that were deposited up to (approximately) 50 years ago. The soil these remains were buried in was very mixed, as many different kinds of soil had been imported for use at the site. It is possible that the soil matrix was more acidic than an average soil context in this part of the world, or could have been subjected to harsher than average environmental

forces, as the remains were far more degraded than some of the following archaeological samples. This higher level of degradation made them ideal testing material for comparison with chemically degraded archaeological samples.

It was on this material that the sample preparation protocols were tested. The drill setup and powder collection procedure was established during this phase of testing. The first trials of the decontamination protocols were conducted on this material, along with the first extractions and testing of one of the three sets of universal primers used throughout the rest of this study. The class breakdown of this test material is summarized in Table 4.

Table 4: Summary of the preservation scale (detailed in Table 2) breakdown for the modern degraded test material.

Preservation Scale	Sample Size	Sample Size Percentage
Class I	8	27%
Class II	12	40%
Class III	6	20%
Class IV	4	13%

These samples were stored in paper field collection bags and had not been washed or processed since collection, so the preparation protocol also had to account for cleaning surface dirt and any associated contaminants off the samples before any further work could be done. It was during this round of testing that general patterns started to emerge. As the drilling progressed through the bone classes the samples became less well preserved, the samples generally became softer and easier to drill although this was not true of all samples. The softness of the bone resulted in the sample being easier to drill, but also increased the chances of mistakes being made and unnecessary damage being done.

It also became evident through the DNA analysis that two applications of bleach with subsequent washes of distilled water was most effective for decontamination and led to fewer contaminated samples. Letting the bone dry completely after the application of liquids was very important, as damp bone powder had a different quality to it when drilling and the resulting bone powder was difficult to weigh accurately. It also became very clear that the bone samples that were more degraded were generally more porous,

and so the bleach and water wash applications soaked into the bone like a sponge and was difficult to remove. Without being able to wash the bleach that soaked into the bone it could continue to degrade the DNA over time and reduce the chances of getting a positive extraction. The more compact, dense bone of the Class I material was easier to work with and probably did not absorb much bleach into the bone matrix.

4.1.4. Test Round 3: Archaeological Samples – Fort D’Epinette

Sixteen unidentified, archaeological samples were randomly selected from the unworked faunal remains of the archaeological site, Fort D’Epinette (HaRc-27, British Columbia) along the Peace River (see Figure 1). Fort D’Epinette, also known as Fort St. John, was occupied from 1806 to 1823 CE, when it was abandoned. The fort was originally established by the North West Company as a fur trading post and was taken over by the Hudson’s Bay Company in 1821. The site was discovered in 1952 and assigned the designation HaRc-27, and then rediscovered 1974 during an archaeological survey directed by Kurt R. Fladmark of SFU. Excavation as part of the SFU field school started the next year and ended in 1976 (Bedard 1990; Burley and Hamilton 1990; Fladmark 1985; Williams 1978). The excavated material was housed by the Department of Archaeology at SFU and has been used for research purposes in the subsequent 40 years.



Figure 1: Map of the location of Fort D'EpINETTE at the confluence of the Peace River and Beatton River, outside the modern town of Fort St John, British Columbia, Canada.

The preservation of the faunal material at this site is excellent with the majority of the remains in very good physical condition. Therefore, this collection yielded very stable test samples. Classes I to IV are all represented within this phase of the testing (see Table 5 for a summary of the material), as well as a range of bone types and thicknesses. At almost 200 years old, this material represents the first test on ancient material. The decontamination protocols and drilling technique were further optimized at this stage, and proved successful enough to proceed onto the first round of case study museum artifacts. In addition to these optimizations, this round of testing also experimented with finding visually unobtrusive locations to drill as the next round of testing would be on the case study museum artifacts. Selecting an unobtrusive place to drill still had to balance the need to maximize compact bone concentrations and avoiding imperfections in the bone which could further destabilize the artifact if agitated.

Table 5: Summary of the preservation scale (detailed in Table 2) breakdown for the Fort D’Epinette test material.

Preservation Scale	Sample Size	Sample Size Percentage
Class I	5	31.25%
Class II	3	18.75%
Class III	5	31.25%
Class IV	3	18.75%

4.1.5. Case Study Museum Samples: SFU Museum of Archaeology and Ethnology

Six modified bone artifacts were selected from the archaeological collections of the SFU Museum of Archaeology and Ethnology with the permission of the museum curator, Dr. Barbara Winter. The artifacts are from four different archaeological sites along the British Columbia coast; McNaughton Island (EITb-10), Anutcix (FaSu-10), Noon’s Creek/Say-mah-mit (DhRq-1), and Crescent Beach (DgRr-1) (see Figure 2). Basic information on these four sites is detailed below.

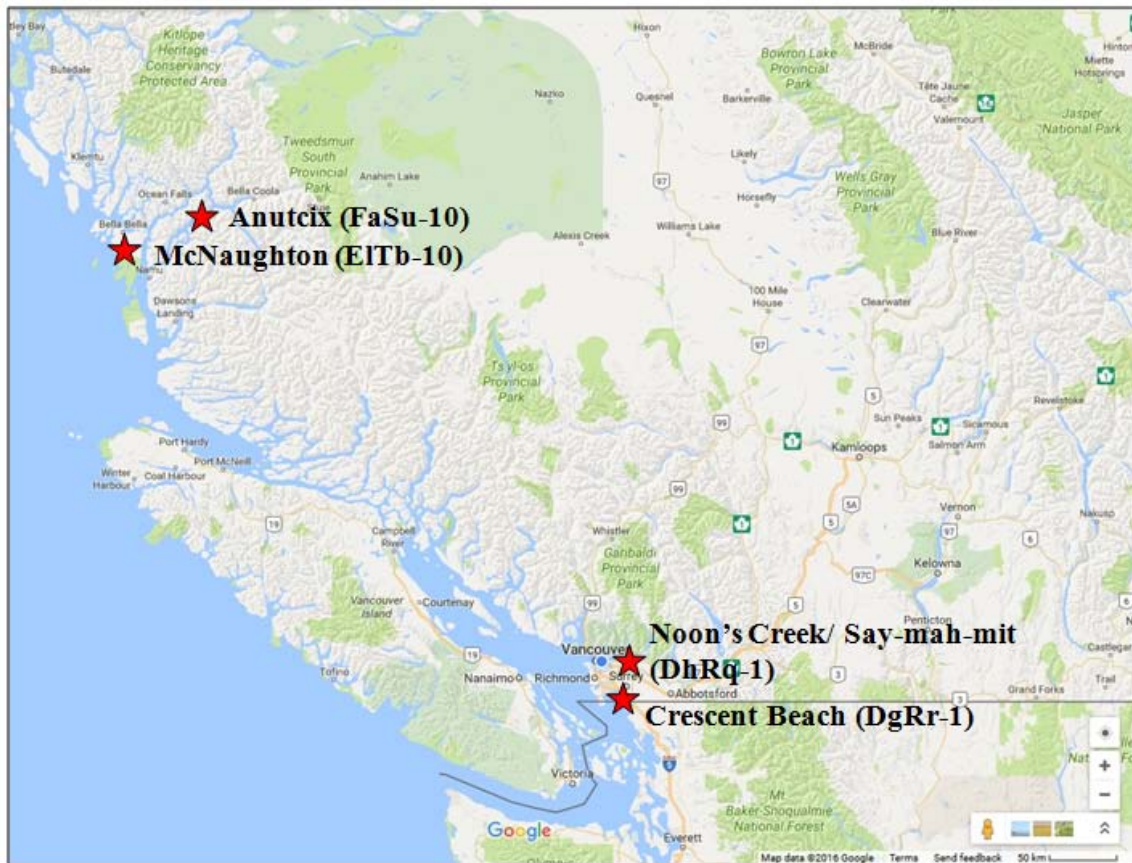


Figure 2: Map of the location of the four SFU Museum sample material archaeological sites in British Columbia, Canada.

The northernmost site represented in this test material is Anutcix (FaSu-10) which is located along the Kwatna River, in the Bella Bella region of the mainland Central Coast. It was excavated in 1972 by Roy L. Carlson of SFU as part of the Department of Archaeology field school (Hobler 1972), and the resulting collection is cared for by the SFU Museum of Archaeology and Ethnology. Two of these artifacts were selected for this round of testing from the SFU museum collection. Anutcix is an ancient village site which characterized the Anutcix Phase, dating to 150-1280 CE (Golder Associates 1999; Hall 1998; Hobler 1972; Pomeroy 1980). The excavated site was largely comprised of large stratified midden deposits, but also contained some habitation features (Hobler 1972; Tobiasz 2015). The Kwatna River is a major salmon river and so it is unsurprising that fish remains made up a large amount of the assemblage, totalling in numbers in excess of 54,000. There were also land and sea

mammals represented in the remains, however land mammals seemed to be favoured (Tobiasz 2015; Pomeroy 1980).

Also in the Bella Bella region is the large site of McNaughton Island (EITb-10), located on the Central Coast near Hunter Island. (Apland 1977; Tobiasz 2015). The research on the site was conducted by two SFU archaeologists; it was first surveyed and tested in 1972 by John A. Pomeroy, and subsequently excavated by Roy L. Carlson in 1974 (Golder Associates 1999; Pomeroy 1980). The resulting collections are cared for by the SFU Museum of Archaeology and Ethnology, and one artifact (a modified bone point) from this collection was selected for analysis in this study. McNaughton Island (EITb-10) is a seasonal (winter/spring) village located on the shore of a lagoon with access to good clam flats, seal hunting and salmon fishing which provided consistent marine subsistence resources (Apland 1977; Pomeroy 1980). The excavated site contained a large four-meter-deep shell midden, habitation features, fish traps and rock art. Radiocarbon dates of the midden deposits range from approximately 500 BCE – 1100 CE (the Kwatna Phase), with historic material on top of the ancient dated material (Golder Associates 1999; Pomeroy 1980). The excavated material included stone tools (chipped, pecked and ground stone), bone and antler harpoons and ornaments, shell beads and tools, and European trade goods (Golder Associates 1999). A very high percentage of the first excavated assemblage was bone and antler material (79% of the 252 artifacts excavated) which indicated a consistent usage of land mammals through time, though this usage gradually shifted towards sea mammals and salmon as time went on (Pomeroy 1980).

Moving down the British Columbia coast, just outside of Vancouver are the next two archaeological sites. Noons Creek, also called Say-mah-mit (DhRq-1), is a seasonal (summer/winter) village located in Burrard Inlet, inland from the present shoreline in Port Moody (Evergreen 2010). Two bone wedge tools were selected for analysis from this site from this site. Noons Creek was first reported as an archaeological site over a century ago by Harlan Smith in 1907 and was subsequently tested in 1950 and 1963, and then excavated in 1971 and 1982. It was first interpreted as a seasonal camp; however, the depth of the cultural deposits suggests a village site (Morin 2015). Noons Creek is a prehistoric shell midden site which has produced radiocarbon dates that span

from 86 BCE to 560 CE (Marpole Phase), however there is archaeological evidence to suggest that the occupation extends into 800-1880 CE (Evergreen 2010; Morin 2015). In 2010 archaeological excavations and assessments were conducted on the site as part of a bridge maintenance project at Noons Creek. This assessment uncovered 24 human bones that belonged to at least two individuals, as well as a number of middens and thermal features which expanded that scope of the site (Stantec 2010).

South of Noons Creek is Crescent Beach (DgRr-1) which is located in Boundary Bay in the southern Fraser River Delta (Ham 1982). It is a large shell midden site that was excavated in 1972 and then again in 1976 and 1977 by the UBC and SFU field schools, though the site was seriously disturbed due to the proximity of city water and sewer lines (Ham 1982; Trace 1981). Dated material, including radiocarbon dates indicated that there was occupation stretching from approximately 1350 BCE – 1450 CE, which includes the Locarno Beach Phase and the Marpole Phase (Evergreen 2010; Ham 1982; Trace 1981). The site was interpreted as a shellfish and herring harvesting camp occupied seasonally in February and March. Artifacts indicate the use of bone and antler fishing tools, and woodworking and hide processing conducted at the site. The single artifact selected for analysis from this site is a bone awl, which is one of several dozen that were excavated (Ham 1982; Trace 1981). Few large mammals were documented at the site as marine resources were favoured, however there were some seasonal preferences for ungulates (such as deer) documented (Ham 1982).

These six samples are mainly comprised of Class I material, but also include Classes II and III (see Table 6 for a summary of the material). The samples also encompass a range of thicknesses, sizes, and bone types. Drill location was very important in this round of sampling as the modified nature of the artifacts presented some additional challenges. Two of the artifacts are bone wedges which could have been used for splitting logs. These artifacts had a modified point at one end and a flattened section at the other which was the result of hammering. Due to this use-wear the natural bone structure was severely modified, and would be very unpredictable to drill into. For these samples it was deemed best to target already exposed surfaces, in order to avoid any cultural modification. The human made modifications to these artifacts may be the features of interest to the museum (for example, if they were to be displayed)

or may be a key identifying feature such as the identification of the date/time period when the artifact was made, or identifying the culture that made it. When possible it is important to avoid altering morphologically or culturally significant features as they can potentially provide other kinds of important information about the object.

Table 6: Summary of class breakdown for SFU’s Museum of Archaeology and Ethnology material.

Preservation Scale	Sample Size	Sample Size Percentage
Class I	3	50%
Class II	2	33.3%
Class III	1	16.7%
Class IV	0	0%

4.1.6. Case Study Museum Samples: Royal Ontario Museum

The Royal Ontario Museum loaned nine late Shang Dynasty (C. 1600-1046 BCE) worked fragmentary bone artifacts from their collections, originally from Anyang (Henan Province, China) for the purposes of this study. The nine artifacts include five oracle bone fragments (some with inscriptions visible, see Table 7), one carved bone vessel fragment, one fragmentary hair pin, and two miscellaneous fragmentary pieces. The oracle bone fragments are of particular interest to the ROM and this study, as the ROM has the largest collection of oracle bones outside China and is interested in seeing the collection utilized for research.

At Anyang in the late Shang Dynasty oracle bones were used in a divination ritual. The diviners would carve their question into the bone and then apply intense heat to the bone which would crack the bone. These cracks would then be interpreted and the answer would be inscribed on the bone. As a result of these inscriptions, the oracle bones bear the earliest known form of ancient Chinese writing (Campbell 2011; Shen 2002; Yuan and Flad 2005). The writing has been studied extensively (Boltz 2000; Bottéro 2004; Demattè 2010; Flad 2008; Keightley 1978, 1989), which has resulted in such information as the complete royal genealogy of the Shang Dynasty and a window into the most significant concerns of the Shang rulers and elite class (Shen 2002; Yuan and Flad 2005).

Four of the five ROM oracle bone fragments have ancient Chinese characters etched into the surface (see Table 7). Huiping Hu, an Associate Professor in the Department of Ancient Texts at the National Library of China (Beijing), agreed to translate the inscriptions. She did point out that these inscriptions only include one or two characters and so a meaningful interpretation of the message that the whole oracle bone inscription would have conveyed is impossible without the rest of the contextual text. Due to the valuable and delicate nature of these artifacts, careful consideration was taken when selecting the drill location to ensure that the scripts would not be impacted.

Table 7: Images and translations of the ROM oracle bone inscriptions.



960.236.581 reads:

"啟" (qi) which means "opening" or "starting".



960.236.583 read:

"九月" (jiu yue) which means "September".



960.236.584 reads:

"卜(bu)...凡(fan)...午(wu)". The meaning of these characters is unclear.



960.236.585 reads:

"辛(xin)...允(yun)" which appears to be an agreement or approval of a missing part of the text.

Although the oracle bones' writing has been studied extensively, there has been no significant DNA work done on the bones outside of the SFU aDNA Laboratory and their collaborators (Brunson 2016). Large, flat bones such as ox scapulae or turtle plastrons were favoured for this divination ritual (Flad 2008; Shen 2002). Although the other four pieces loaned by the ROM are likely to be from large land mammals, the five oracle bone fragments are likely to be turtle plastrons. As these plastrons were likely fired in ancient time they pose a particular challenge for DNA recovery, and as they are very thin they posed the greatest challenge for the drilling technique. These nine pieces represent the last round of testing for this research.

4.2. Pre-Drilling Sample Preparation

4.2.1. Morphological Based Species Identification

For all unknown bone samples morphologically based species identifications were conducted by Shea Henry (PhD candidate) at SFU using the SFU zoological comparative collections. This information was important as it gave a baseline assessment of what animals may be represented in the sample material which aided in primer selection. It was possible to estimate the efficacy of morphological based analysis by comparing them to the DNA based species identification. However, the main strength of including both morphological and DNA based species analysis in this project is to illustrate the number of archaeological remains that are simply too fragmentary or modified to identify morphologically which can be identified genetically, with great confidence. The final data is compared in the “Results” chapter.

4.2.2. X-Ray Imaging

To avoid any unnecessary damage to the internal structural integrity of the samples, x-rays were taken of all of the archaeological sample material. All the pre-drilling x-rays were done with the SFU Department of Archaeology x-ray machine (H.G. Fischer, Inc., Chicago, Illinois, USA). With these images it was possible to assess the internal structure of the samples and see any internal cracks, fractures or imperfections that, if disturbed with the drill, may destabilize the bone sample and cause unintended long term damage.

In addition to assessing for pre-existing damage, the x-rays helped to visualize which areas of each bone sample had the greatest density of solid, compact bone. Compact bone produces the greatest amount of powder as compared to trabecular bone, and contains collagen which is positively correlated with DNA (Campos et al 2012). It is also hypothesized that drilling into compact bone would be more stable than drilling into trabecular bone. These x-rays were used in the drill site selection process.

X-ray images were also taken after the samples were drilled. Unfortunately, the SFU Department of Archaeology x-ray machine broke in the interim, and so the post-

drilling x-rays were taken using the SFU Department of Earth Sciences x-ray machine (SY-31-100 Portable X-Ray, Soyee Products Inc, New York, USA) and visualized with a Kodak VITA Computer Radiography System (Rochester, New York, USA) and processed by Omnivue QC (Genesis Digital Imaging, Los Angeles, California, USA). The comparison of the x-rays taken during sample preparation and those taken post analysis would reveal any unintended damage or bone instability that could be created as a result of the drilling process. The comparisons of these x-rays are not only an important step in avoiding unnecessary damage but also in assessing the degree of damage that may be caused by this technique. For a full side by side comparison see Appendix B.



Figure 3: Pre-drilled x-ray images showing the internal structure of the bones from a selection of the Fort D'EpINETTE archaeological samples.

4.2.3. Drill Site Selection

The drill site for each bone sample was selected in order to maximize bone powder, and minimize internal and external damage. The x-ray images in combination with careful visual assessment for smaller cracks and imperfections not captured by the x-rays led to the selection of the optimal drilling location and depth per sample.

Selecting the drill sites for the museum artifacts includes the extra step of consultation with the appropriate museum curator or collection manager. As the curator may have special concerns such as the “displayability” of the artifact after drilling they should be consulted so that all concerns regarding the artifacts are addressed appropriately. Selecting the drilling site for the artifacts is then a matter of balancing the optimal drilling location as suggested by the x-ray imaging and visual assessment, with any concerns the curators may have. During this project, the SFU Museum artifacts’ drill locations were selected after a discussion with the museum Director, Dr. Barbara Winter and her approval of the proposed locations. The curator of the ROM’s artifacts, Dr. Chen Shen, opted to defer to our judgment and pre-approved the selected locations for their loaned artifacts based on our recommendations.

4.3. Drilling Technique

4.3.1. Drill Information

The drill used throughout this research project was a NSK Ultimate XL drill (renamed NSK Forza L50K). The drill has a speed range of 1,000 – 40,000 rpm and useful accessories such as a foot pedal and a handpiece stand. The full range of speeds were explored in the first and second rounds of sample tests. Any speed above 2,000 rpm was found to be too aggressive and hard to control (ie. kickback creating external damage), and the high speeds overheated the bone and produced smoke and a burning smell. The heat created as a result of the friction from the drill actually degrades any surviving DNA further, and may hinder chances for aDNA recovery (Pichler *et al.* 2001; Rohland and Hofreiter 2007a). As a result, all archaeological materials were drilled at 1,000 rpm, the lowest speed offered on this model.

The drill bits used in this study were round friction grip carbide bits, which allowed for great versatility. Five drill bit sizes were used; 0.8mm, 1.0mm, 1.4mm, 1.8mm and 2.3mm (Figure 4). The largest drill bit of 2.3mm (not pictured) was experimented with, but eventually deemed unnecessarily large for this study and so it was excluded early in the testing rounds.

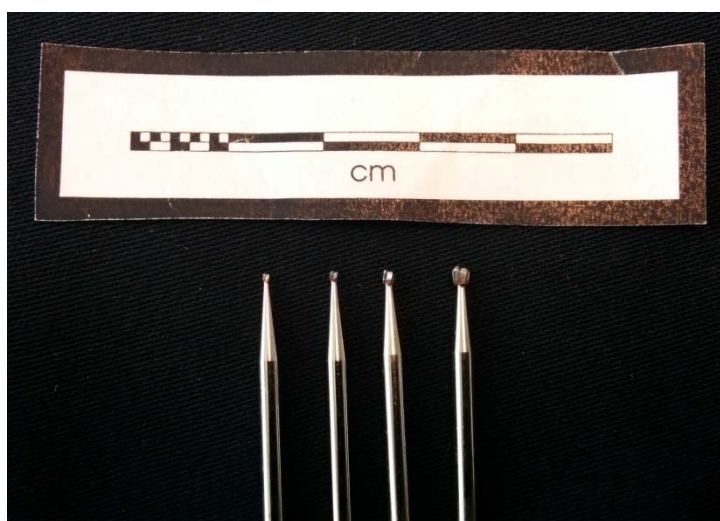


Figure 4: Round friction grip carbide bits, (L-R) 0.8mm, 1.0mm, 1.4mm, 1.8mm from Burs for Carving (Scottsdale, Arizona, USA).

4.3.2. Drilling Setup and Technique

All drilling, with the exception of the Anyang samples which were drilled in the dedicated Ancient DNA Laboratory, were drilled in a multi-purpose laboratory at SFU. Prior to any drilling work all working surfaces in the lab were cleaned and bleached. The drill machine was placed on the lab bench, and was controlled by the seated researcher by the foot pedal. Disposable sterile bench pads, wipes, and weight boats were used for each sample so there would be no cross-contamination between samples or from their resulting powder or soil. A lab coat and gloves were also used for all sampling.

As noted above, round carbide bits were used for this study. These bits afford a greater amount of control and maneuverability than other shaped bits would (such as a cone shaped drill bit), which reduces the opportunity for any unplanned damage to the internal or external surface of the samples. The technique involves using two drills bits per sample; one larger drill bit to make the initial external hole, and then a drill bit two

sizes smaller to carve out the interior of the bone (such as starting with a 1.8mm bit and switching to a 1.0mm bit). This technique maximizes the bone powder available for collection while still minimizing the visible damage on the outside of the sample.

4.3.3. Decontamination Protocol

Through systematic testing, an effective decontamination protocol was established which leaves the samples visually, chemically and structurally intact and we hypothesize that this will not affect long term preservation. As this method is tailored for use on museum artifacts, it is not possible to decontaminate the sample by abrading the surface to expose the uncontaminated inner compact bone or by soaking the entire artifact in a bleach solution for an extended period of time (Cooper and Poinar 2000; Handt *et al.* 1994; O'Rourke *et al.* 2000; Yang and Watt 2005; Yang *et al.* 2005). Therefore, the largest challenge for this technique to overcome is the effective decontamination of the samples prior to drilling.

Once all the pre-drilling sample preparation was complete (morphological analysis, x-ray imaging, and drill site selection), the sample was wrapped in parafilm, exposing only the drill location. If the sample was delicate (Classes II – IV) it was wrapped with clean tissues (Kimwipes) first, so that the parafilm couldn't bond to the bone and strip off any fragile surface pieces when removed. A cotton swab soaked in 100% bleach (5% sodium hypochlorite) was then applied to the exposed drill location to decontaminate the surface of the bone. After approximately 30 seconds a cotton swab soaked in water was applied to wash off the bleach. This step was conducted at least twice for each sample and then the sample would be left to dry for 30-60 minutes depending on the bone.

Once the surface decontaminants were removed the sample was drilled with two drill bits. The powder fell into a new weight boat and was transferred into a 15 mL tube. For the first four rounds of testing the drill bits were reused, and so effective decontamination was necessary to ensure no cross contamination between samples. Each drill bit was scrubbed with detergent to remove any macro particles of bone powder. Then the drill bits were soaked in 100% bleach (5% sodium hypochlorite) for

several minutes. Once bleached the drill bits were washed in a double distilled water bath to remove any bleach that might corrode the metal, and then dried.

4.4. DNA Analysis

4.4.1. Contamination Controls

All DNA extractions and PCR setups were carried out in the dedicated Forensic DNA Laboratory and Ancient DNA Laboratory, each with separate rooms for each step of the analysis (sample preparation, DNA extraction, and PCR setup). Strict contamination protocols (Cooper and Poinar 2000; Gilbert *et al.* 2005; Yang and Watt 2005) used for the study of ancient DNA were followed, for example bleach was used to destroy surface contaminants and multiple blank and negative extraction samples were also set up alongside the ancient DNA samples to confirm the absence of contaminants in the samples.

PCR amplifications and subsequent work involving PCR products were conducted in a separate laboratory. The PCR laboratory and the ancient DNA laboratory are physically separated from each other and are situated in two different buildings on the university campus to further reduce the possibility of contamination.

4.4.2. DNA Extraction

The bone powder produced by the drilling method was collected in 15 mL tubes. The normal lab protocol is to soak each sample in 100% bleach (5% sodium hypochlorite) solution for 3-10 min to remove any leftover contamination. However, a test conducted for this research on a sample from the modern degraded test material indicated that directly bleaching the bone powder in the lab was too harsh and eliminated all the targeted aDNA along with any potential contaminants. Ten drilled bone test samples (TS021-TS030) were taken from a single modern degraded sample (Figure 5 [A]) in order to reduce the variables present in this experiment. Samples TS021-TS025 were extracted without the use of bleach, while TS026-TS030 were extracted after being soaked with bleach for 1-2 minutes. All five samples that were extracted without the use

of bleach yielded positive aDNA sequences, while those that were exposed to the bleach solution yielded no DNA sequences (Figure 5 [B]). As all ten samples are from the same source, it is safe to assume that they all contained the same amount of DNA and that the negative results in samples TS026-TS030 are the result of the bleach treatment.

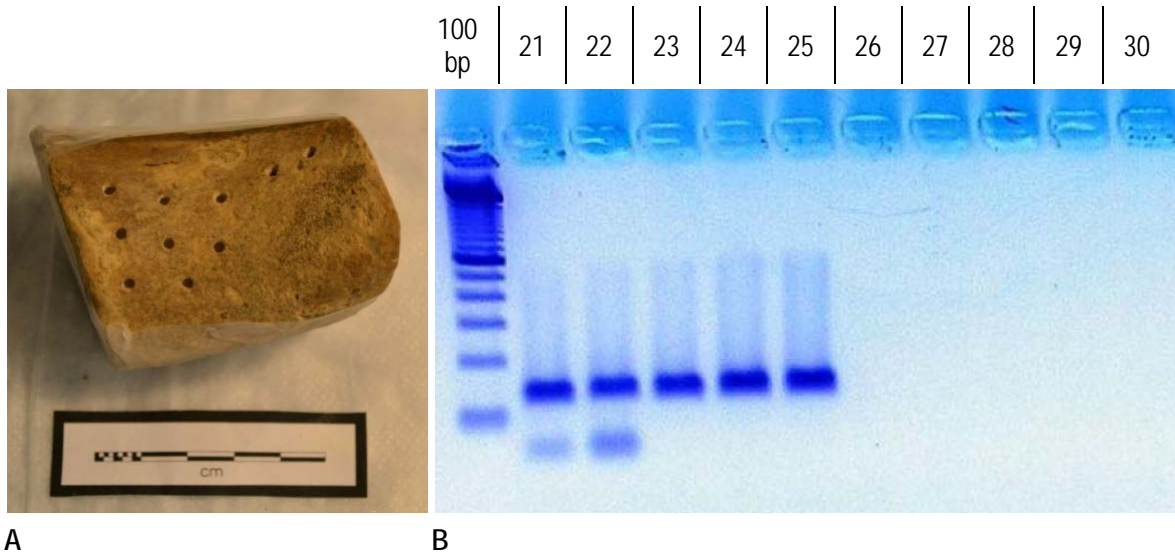


Figure 5: Ten samples to test the effect of directly bleaching the bone powder during the extraction process: (A) ten samples drilled from the same modern degraded test sample (B) After 60 cycles of PCR the amplicons were visualized with SybrGreen on a 2% agarose gel. 100 bp represents the 100 bp ladder. Samples TS021-025 were extracted without bleach being applied directly to the bone powder, and samples TS026-030 were extracted with bleach applied directly to the bone powder for 1-2 minutes.

A modified silica-spin column method DNA (Yang *et al.* 1998) was employed to extract the. Added to the 15 mL tubes containing the bone powder was 3 mL (2 mL for Anyang samples) of a lysis buffer composed of EDTA (0.5 M, pH 8.0), SDS (0.25%) and proteinase K (0.5 mg/mL) designed to break down the matrix of bone and release the aDNA. This solution was then incubated overnight in a rotating hybridization oven (Model 6243, Thermo Fisher Scientific, Nepean, Ontario, Canada) at 50°C. Following incubation, the samples were centrifuged (Model 5702, Eppendorf, Hamburg, Germany) at 4,400 rpm for 5 minutes to concentrate the remaining powder at the base of the tube. After centrifugation, 1 mL of supernatant was transferred to an Amicon centrifugal filter for each sample. The Amicons were then centrifuged at 4,400 rpm for 40-60 minutes until the supernatant was reduced to less than 100 µL.

The concentrated supernatant was then purified through the use of QIAquick spin columns, first by adding 500 µl of PB Buffer (QIAGEN, Hilden, Germany). The spin columns were centrifuged (Model 5424, Eppendorf, Hamburg, Germany) for one minute at 13,000 rpm to bind the DNA to the silica membrane. The spin column was then transferred to a new 2 mL collection tube, and each column was washed with 500 µl PE Buffer (QIAGEN, Hilden, Germany) and then spun down for one minute at 13,000 rpm. The column was again transferred to a new 2 mL collection tube, and each column was washed with a further 300 µl PE Buffer (QIAGEN, Hilden, Germany) and then spun down for three minute at 13,000 rpm. The column was transferred to a new conical 2 mL collection tube and 100 µl EB Buffer was (QIAGEN, Hilden, Germany) added. Each sample was then incubated in a heat block (Isotemp, Thermo Fisher Scientific, Nepean, Ontario, Canada) at 70°C for 5 minutes, or until the membrane began to drip. The samples were then centrifuged for one minutes at 13,000 rpm and the resulting elution was transferred to a 0.5 mL collection tube. Another 100 µl of EB Buffer (QIAGEN, Hilden, Germany) was added to the sample column and collection tube, and this last step was repeated to produce the second elution which was then transferred to the second 0.5 mL tube. The first elution will have more aDNA but will also hold the possibility of more inhibitors, whereas the second elution is cleaned again and may therefore have less inhibitors but also less aDNA. Both of the elutions were stored at -20°C.

4.4.3. PCR Amplification

The PCR process takes advantage of DNA's double-stranded structure which can be broken into single strands with the application of heat. Through the use of primers and thermal cycling (repeating cycles of heating and cooling) it is possible to take one molecule of DNA and artificially synthesise billions of DNA fragment copies that can then be sequenced. (Bartlett and Stirling 2003; Matisoo-Smith and Horsburgh 2012). The extracted aDNA is added to this mixture and is then run through the PCR process using a thermal cycler. Mitochondrial DNA (mtDNA) was chosen as the DNA marker for this study due to its high copy number per cell. Standard PCR was used to assess whether or not the samples contained amplifiable aDNA. The PCR amplifications were conducted in a Mastercycler Personal Thermal Cycler (Eppendorf, Hamburg, Germany).

In the field of aDNA, the DNA that is extracted is very degraded and is usually around 100-500 bp in length, therefore the sequences that are targeted for PCR amplification have to be short (O'Rourke *et al.* 2000; Willerslev and Cooper 2005). To target these sequences, two PCR primers (forward and reverse) are designed to flank the targeted sequence. The primers are added to a reaction buffer along with dNTPs (the nucleotides that will build the new strands), magnesium (catalyst for the Taq enzyme to start the chain-building process), BSA proteins, and a thermostable Taq DNA polymerase (Brown and Brown 2011; Matisoo-Smith and Horsburgh 2012; O'Rourke *et al.* 1996). The primers of varying lengths used in this study are summarized in Table 8.

Table 8: Primers used in the PCR amplifications.

Primer Name	Sequences (5' – 3')	Amplicon	Target Region	Species	Source
L1269	CATGAAGCACGCACACACCG	117 bp	12S Gene rDNA	Universal Mammal	Rollo <i>et al.</i> 2002
H1346	CCAGTATGCTTACCTTGTTAC		mtDNA		
L1085	CCCAAAGTGGGATTAGATACCC	215 bp	12S Gene rDNA	Universal Vertebrate	Kitano <i>et al.</i> 2006
H1259	GTTTGCTGAAGATGGCGGTA		mtDNA		
L14816	CCATCCAACATCTCCGCATGATGAAA	300 bp	Cytb mtDNA	Universal Vertebrate	Parson <i>et al.</i> 2000
H15173	CCCCTCAGAATGATATTTGGCCTCA				

The first elution was processed in a 30 µl reaction volume containing 50 mM KCl, 10 mM Tris-HCl, 2.5 mM MgCl₂, 0.2 mM dNTP, 1.0 mg/mL BSA, 0.3 µM of each primer, 3.0 µl DNA sample and 2.50 U of AmpliTaq Gold™. This reaction mixture (pre-mix) is tailored for each PCR sample setup and contains all of the buffers, nucleotides and BSA required for the PCR to be successful.

PCR was run at 60 cycles at a high temperature of 95°C for 30 sec (denaturing) to break the bonds and separate the DNA into single strands, 52°C for 30 sec so the primers can anneal to the now single stranded DNA, and 70°C for 40 sec so the primers extend and the synthesis of the new DNA strand is complete. An initial denaturing was performed at 95°C for 12 min to effectively activate the polymerase. Five microliters of PCR product were separated by electrophoresis on a 2% agarose gel immersed in 0.5x TBE loading buffer (100V for thirty minutes). A 100 bp ladder (Invitrogen Life

Technologies, Carlsbad, California, USA) was used for each gel. The pcr product was visualized using SYBR Green™ (Invitrogen Life Technologies, Carlsbad, California, USA) staining on a Dark Reader Box (Model DR46B, Clare Chemical Research, Dolores, Colorado, USA) and sequenced uni-directionally by Eurofins Genomics (Louisville, Kentucky, USA).

4.4.4. Species Identification

The resulting sequences once returned from Eurofins were edited using ChromasPro (<http://www.technelysium.com.au>). These edited sequences were then cross-referenced against GenBank through a BLAST (Altschul *et al.* 1990) search. This search was enough to obtain taxonomic identifications at the species or genus level which are detailed in the following chapter.

Chapter 5.

Results

5.1. Morphological Preservation Scale

Using a morphological preservation scale to inform the sample selection process guaranteed a full range of morphologically preserved testing material for this study. The material for this study was assigned into classes I through IV based on observable features such as luster, porosity, cracking and density (see Table 2). As the drilling progressed through the four classes of material, generally the bone material became softer, more porous and easier to drill. The porosity of the bone became a concern during the decontamination phase of the sample preparation. The bleach used for the sample decontamination easily penetrated the bone matrix like a sponge. Thus it was difficult to wash with water and remove the degrading effect of the bleach from the inside of the bone, which could continue to degrade the DNA over time. Generally, the more compact, dense bone of the Class I material did not absorb much bleach into the bone matrix which made it easier to remove.

The softness of the bone in the later classes also posed a challenge for drilling. With very soft bone that turns to powder quickly and without any resistance it is very easy to make a drilling mistake such as removing too much bone, or drilling too close to an edge which can cause a fracture or break. With soft bone it is also possible to make the entry hole into the bone larger than intended. Thus it was important to proceed through several rounds of testing conducted on unworked remains before progressing onto unique museum artifacts.

The preservation scale was very helpful in assessing this technique; however, no patterns emerged with regard to morphological preservation and DNA preservation.

There are studies to suggest that DNA preservation is correlated with morphological preservation, specifically collagen preservation (Götherström *et al.* 2002; Haynes *et al.* 2002), however there was no evidence of such a correlation in this research project. The samples from each class type amplified with comparable success. As there are many factors that could affect DNA preservation, such as water or oxygen, acidity, salt content, radiation, or age (Campos *et al.* 2012), it is possible that consistency among these factors weighed more heavily in the preservation of the DNA than the morphological preservation.

5.2. Species Identification

5.2.1. Morphological Analysis

The morphological analysis conducted after the samples had been selected confirmed that the majority of the samples were land mammals, and therefore within the selection criteria. However, the results of the analysis (detailed in Tables 9-12) indicate that the samples were either too fragmented or too modified, with too few distinguishing characteristics to speciate in 54% of the samples.

Due to the often fragmentary and deteriorated state of archaeological remains it can be a challenge to accurately assign species identification based on morphological characteristics (Speller *et al.* 2005; Yang *et al.* 2004; Yang *et al.* 2005). The results from this study's morphological comparison lend weight to the importance of DNA based species identification in archaeological contexts, and highlight the value of its accuracy.

5.2.2. Modern Degraded Samples

Of the 30 samples selected for this round of testing, 21 were subsampled for analysis with 17 of them producing reliable DNA sequences which led to species identifications. This is an 81% success rate in the first round of analysis. Three of the samples did not produce amplifiable results, and one sample produced an amplifiable result which turned out to be bacterial contamination. The samples were all amplified

using universal mammal primers (L1269/H1346) for short 100 bp fragments (see Table 8) and were identified using BLAST searches using the GenBank database.

In tandem with the DNA analysis, morphological analysis was conducted on all of the samples for comparison purposes. Of the total 30 samples, 15 of them (50%) were too fragmented to speciate (see Table 9). Of the 17 samples that yielded successful DNA based species identifications, 11 of them (65%) were too fragmented to speciate morphologically. Of the remaining six samples that were identifiable morphologically and were successfully DNA tested, four were identified correctly for a 67% success rate.

A DNA amplification success rate of 81% in the first round of testing indicated that the decontamination protocol being tested was very successful, and just needed to be optimized to increase the success rate. In this round of testing it was also proved possible to obtain a successful species identification from less than 10 mg of bone powder. The smallest amount used successfully in this round of testing was TS 005 with 7 mg of bone powder. The smaller the amount of bone powder necessary to obtain a reliable sequence means that less damage needs to be done to the object which makes this technique less impactful.

Table 9: Comparison of the morphological and DNA based species identification on the modern degraded test samples. All the species identified via the DNA based analysis are local to the Port Coquitlam (BC) area except for the moose and white-tailed deer.

Sample Name	Morphological Species Identification	DNA Species Identification	Powder Weight (mg)
TS 001	Cow	Unsuccessful Amplification	-
TS 002	Pig	Cow (<i>Bos taurus</i>)	42
TS 003	Goose	Not Selected for Analysis	21
TS 004	Medium Mammal, not enough to speciate	White-tailed deer (<i>Odocoileus virginianus</i>)	-
TS 005	Medium Mammal, not enough to speciate	Cow (<i>Bos taurus</i>)	7
TS 006	Medium Mammal, not enough to speciate	Not Selected for Analysis	8
TS 007	Pig	Not Selected for Analysis	12
TS 008	Cow	Cow (<i>Bos taurus</i>)	7

Sample Name	Morphological Species Identification	DNA Species Identification	Powder Weight (mg)
TS 009 – TS010	Large Mammal, not enough to speciate	Cow (<i>Bos taurus</i>)	14
TS 011	Cow	Cow (<i>Bos taurus</i>)	14
TS 012	Large Mammal, not enough to speciate	Moose (<i>Alces alces</i>)	9
TS 013	Pig	Not Selected for Analysis	19
TS 014	Medium Mammal, not enough to speciate	Sheep (<i>Ovis aries</i>)	43
TS 015	Cow	Cow (<i>Bos taurus</i>)	10
TS 016	Large Mammal, not enough to speciate	Cow (<i>Bos taurus</i>)	121
TS 017	Deer	Not Selected for Analysis	17
TS 018	Large Mammal, not enough to speciate	Not Selected for Analysis	23
TS 019	Deer	Cow (<i>Bos taurus</i>)	12
TS 020	Cow	Not Selected for Analysis	19
TS 021 – TS 030	Large Mammal, not enough to speciate	Cow (<i>Bos taurus</i>)	15
TS 031	Cow	Not Selected for Analysis	8
TS 032	Large Mammal, not enough to speciate	Unsuccessful Amplification	3
TS 033	Pig	Unsuccessful Amplification	3
TS 034	Pig	Not Selected for Analysis	13
TS 035	Large Mammal, not enough to speciate	Cow (<i>Bos taurus</i>)	8
TS 036	Large Mammal, not enough to speciate	Cow (<i>Bos taurus</i>)	12
TS 037	Pig	Wild boar/Pig (<i>Sus scrofa</i>)	8
TS 038	Medium Mammal, not enough to speciate	Bacterial Contamination	5
TS 039	Large Mammal, not enough to speciate	Cow (<i>Bos taurus</i>)	13
TS 040	Large Mammal, not enough to speciate	Cow (<i>Bos taurus</i>)	12

5.2.3. Archaeological Samples – Fort D’Epinette

All 16 samples selected for this round of testing were processed for DNA analysis, with 14 of them producing reliable DNA sequences which led to species identifications. This is an 88% success rate and an improvement on the 81% success rate from the previous round of testing. The remaining two samples did produce amplifiable DNA but after sequencing it turned out to be human contaminant DNA. The samples were amplified using three different universal mammal and universal vertebrate primers (see Table 8) ranging from 100-300 bp (L1269/H1346, L1085/H1259, and L14816/H15173) and were identified using BLAST searches.

Morphological analysis was also conducted on all of these samples for comparison purposes. Of the total 16 samples, nine of them (56%) were too fragmented to speciate (see Table 10). Of the 14 samples that yielded successful DNA based species identifications, eight of them (57%) of them were too fragmented to identify morphologically. Of the remaining six samples that were identifiable morphologically and were successfully DNA tested, four were identified correctly for a 67% success rate and equal to that of the previous round of analysis.

A success rate of 88% represents an improvement on the first round of testing and indicated that the optimizations made to the sampling and DNA extraction protocol were successful. However, the presence of human contamination indicated that the protocol was not yet 100% effective for decontamination. In this round of testing it was also possible to decrease the amount of bone powder necessary to obtain a species identification. The smallest amount used successfully in this round of testing was sample 305 with 3 mg of bone powder. With the small amount of bone powder necessary for a positive amplification and a consistent success rate it was possible to confidently move on to the case study museum artifacts in the next round of testing.

Table 10: Comparison of the morphological and DNA based species identification on the Fort D’EpINETTE archaeological samples.

Sample Name	Morphological Species Identification	DNA Species Identification	Powder Weight (mg)
301	Elk	Red deer/Elk (<i>Cervus elaphus</i>)	13
302	Marine mammal	Moose (<i>Alces alces</i>)	18
303	Not enough to speciate	Red deer/Elk (<i>Cervus elaphus</i>)	18
304	Not enough to speciate	Moose (<i>Alces alces</i>)	16
305	Not enough to speciate	Moose (<i>Alces alces</i>)	3
306	Not enough to speciate	Red deer/Elk (<i>Cervus elaphus</i>)	13
2957	Elk	Red deer/Elk (<i>Cervus elaphus</i>)	21
3095	Medium mammal, not enough to speciate	Contamination – Human (<i>Homo sapiens</i>)	2
4057	Medium mammal, not enough to speciate	Gray wolf (<i>Canis lupus</i>)	11
6352	Canide (Canine)	Gray wolf (<i>Canis lupus</i>)	23
6858	Canide (Canine)	Gray wolf (<i>Canis lupus</i>)	14
27:2042	Bear, could be human	Contamination – Human (<i>Homo sapiens</i>)	13
27:709	Medium mammal, not enough to speciate	Gray wolf (<i>Canis lupus</i>)	12
A25E1	Young animal, not enough to speciate	Moose (<i>Alces alces</i>)	16
A45E10	Large mammal, not enough to speciate	Moose (<i>Alces alces</i>)	15
A7-2	Elk	Moose (<i>Alces alces</i>)	18

5.2.4. Case Study Museum Samples: SFU Museum of Archaeology and Ethnology

There were six artifact samples selected from the SFU museum and all six were processed for DNA analysis. All six samples produced reliable DNA sequences which led to species identifications which is a 100% success rate (see Figure 6). Again, this round of testing improved upon the previous two rounds of testing. The samples were amplified using three different universal mammal and universal vertebrate primers (see Table 8) ranging from 100-300 bp (L1269/H1346, L1085/H1259, and L14816/H15173) and were identified using BLAST searches (Figure 7).

The BLAST search for the sample 323 sequence actually came back with three equally likely species identifications (each with 90% confidence): Indian hog deer (*Axis porcinus*), chital (*Axis axis*), and red deer (*Cervus elaphus*). Both the Indian hog deer and the chital are deer species native to India, whereas red deer are native to British Columbia. On this geographic basis the red deer was identified as the correct species identification.

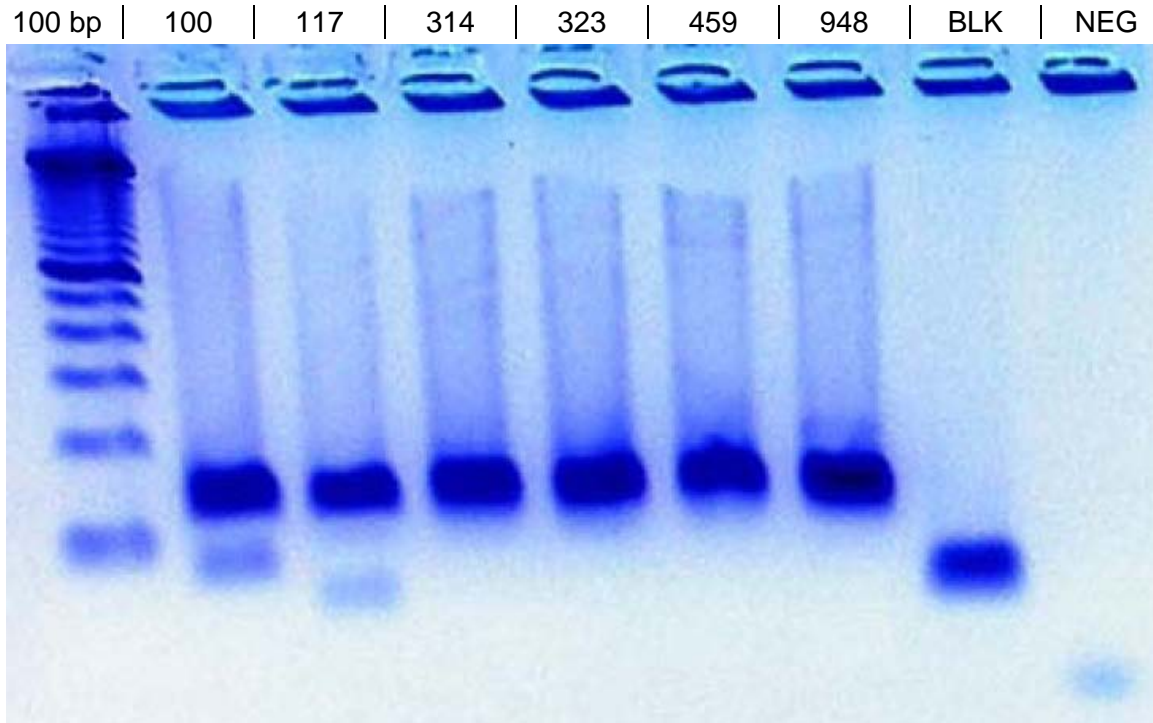


Figure 6: Successful amplification of all six SFU Museum of Archaeology and Ethnology artifact samples. After 60 cycles of PCR the amplicons were visualized with SybrGreen on a 2% agarose gel. 100 bp represents the 100 bp ladder.

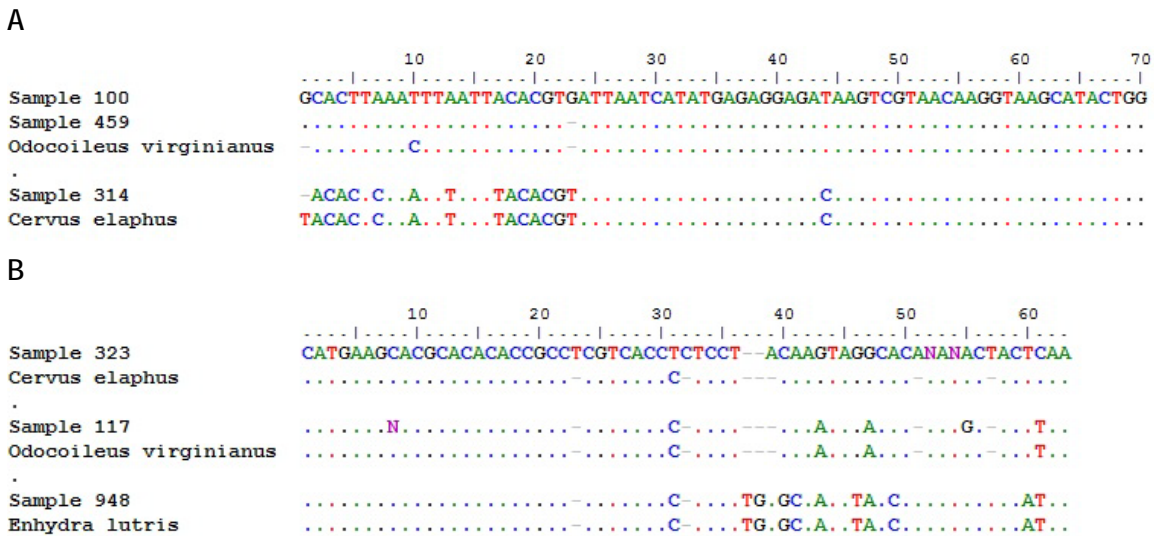


Figure 7: (A) Multiple-alignment of ancient 12S Gene sequences from samples 100, 314, and 459 from the SFU Museum of Archaeology and Ethnology and GenBank reference sequences. These samples were sequenced using L1269 primer. GenBank accession numbers: *O. virginianus* JN632673.1; *C. elaphus* KU942399.1. (B) Multiple-alignment of ancient 12S Gene sequences from samples 117, 323, and 948 from the SFU Museum of Archaeology and Ethnology and GenBank reference sequences. These samples were sequenced using H1346 primer. GenBank accession numbers: *C. elaphus* KU942399.1; *O. virginianus* JN632673.1; *E. lutris* EF472272.1. The dots indicate identical base pairs when compared to each other. The sequences have been edited to remove primer sequences.

Morphological analysis was also conducted on all six of these samples. Half of these samples were either too fragmented or too modified to morphologically speciate (see Table 11). Of the three samples that were identifiable morphologically as well as successfully DNA tested, one was identified correctly which represents a 33% success rate. This success rate is less than half that of the two previous rounds of testing.

A success rate of 100% represented an improvement on the first two rounds of testing and also indicated a successfully optimized decontamination and extraction protocol and drilling method. The bone powder used for these six case study samples averaged around 15 mg, with the smallest amount of powder weighing 10 mg. With a 100% success rate the technique was deemed reliable enough to move on to fragile ancient Chinese material on loan from the ROM.

Table 11: Comparison of the morphological and DNA based species identification on the SFU Museum of Archaeology and Ethnology samples.

Sample Name	Morphological Species Identification	DNA Species Identification	Powder Weight (mg)
100	Deer	White-tailed deer (<i>Odocoileus virginianus</i>)	15
117	Elk	White-tailed deer (<i>Odocoileus virginianus</i>)	16
314	Too modified to speciate	Red deer/Elk (<i>Cervus elaphus</i>)	20
323	Too modified to speciate	Red deer/Elk (<i>Cervus elaphus</i>)	18
459	Not enough to speciate	White-tailed deer (<i>Odocoileus virginianus</i>)	10
948	Wolf	Sea otter (<i>Enhydra lutris</i>)	14

5.2.5. Case Study Museum Samples: Royal Ontario Museum

There were nine artifact samples selected by Dr. Chen Shen of the ROM for use in this study. These samples were amplified using two different universal mammal and universal vertebrate primers (see Table 8) ranging from 100-200 bp (L1269/H1346 and L1085/H1259). The resulting amplifications were identified using BLAST searches.

Morphological analysis was also conducted on all nine of these samples. Six of them (67%) were either too fragmented or too modified to speciate (see Table 12). The remaining three were identified as turtle, however they failed to produce amplifiable DNA to confirm the species identification. Overall this round of DNA testing had a 11% success rate.

Table 12: Comparison of the morphological and DNA based species identification on the ROM samples.

Sample Name	Morphological Species Identification	DNA Species Identification	Powder Weight (mg)
960.236.41	Too modified to speciate	Contamination – Human (<i>Homo sapiens</i>)	14
960.236.224	Large mammal, too modified to speciate	Contamination – Human (<i>Homo sapiens</i>)	10
960.236.383.1	Large mammal, not enough to speciate	Cow (<i>Bos Taurus</i>)	16
960.236.482	Medium mammal, not enough to speciate	Contamination – Human (<i>Homo sapiens</i>)	13
960.236.581	Turtle	Contamination – Human (<i>Homo sapiens</i>)	12
960.236.582	Turtle	Contamination – Human (<i>Homo sapiens</i>)	10
960.236.583	Turtle	Contamination – Human (<i>Homo sapiens</i>)	13
960.236.584	Not enough to speciate	Contamination – Human (<i>Homo sapiens</i>)	7
960.236.585	Not enough to speciate	Unspecific Ungulate	7

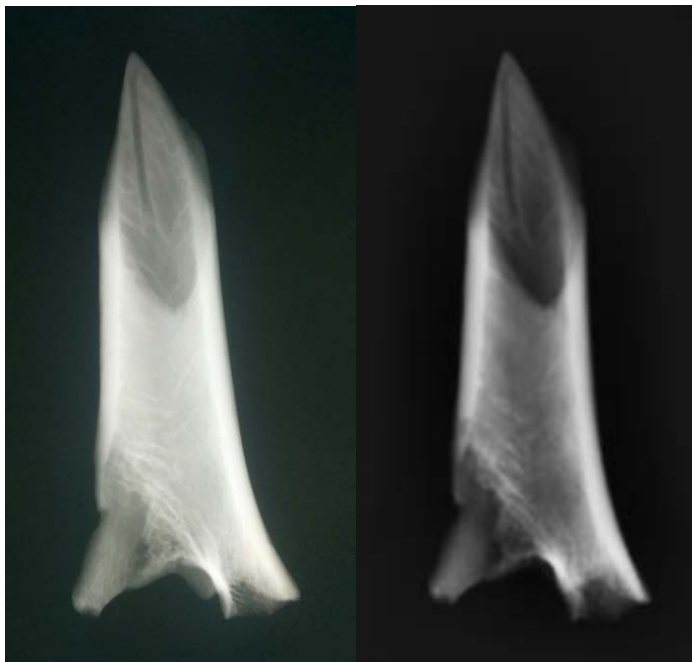
5.3. X-Ray Imaging

To avoid any unnecessary damage to the internal structural integrity of the samples, x-rays were taken of all of the archaeological sample material during the preparation phase. These x-rays were used in the drill site selection process. After the samples were drilled and the bone powder samples collected for analysis, the samples were x-rayed again. These x-rays were compared to the original x-rays taken during the sample preparation phase to assess any damage caused by the minimally invasive drilling protocol.

The comparison of these x-rays showed no significant damage done to the samples and artifacts. There were no additional cracks, fissures or areas of destabilization that appear on the later x-rays that weren't observed in the earlier x-rays. No additional, unnecessary damage was produced as a result of this technique. It is also

predicted that the drilling did not destabilize the samples and artifacts in any significant way, and so it is unlikely that this technique has affected the object's long term preservation. An example from each round of testing that underwent x-ray imaging is provided in Table 13⁵.

Table 13: An example comparison of the pre-drilled x-rays (on the left) and the post-drilled x-rays (on the right) from each of the three rounds of testing that underwent x-ray imaging.

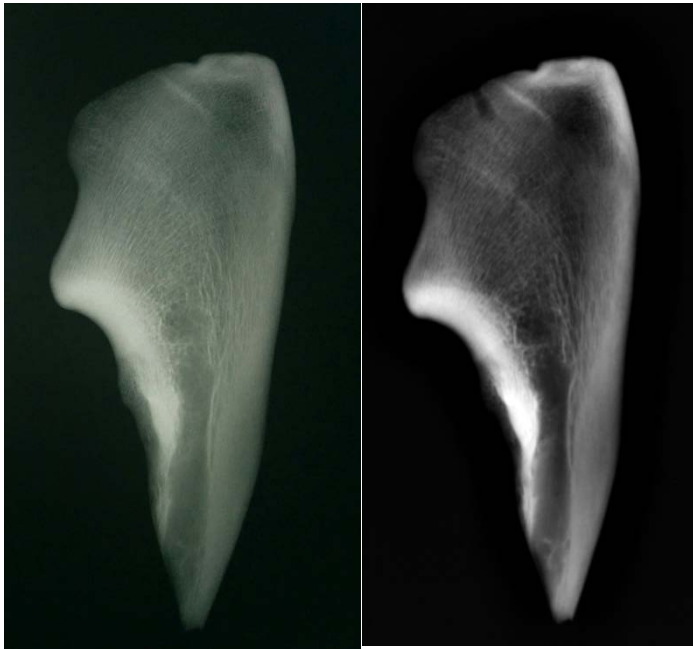


Fort D'EpINETTE

Sample 4057 - Gray wolf (*Canis lupus*)

Unmodified bone fragment

⁵For a full side by side comparison of each pre and post-drilled sample see Appendix C.



SFU Museum of Archaeology
and Ethnology

Crescent Beach (DgRr-1)

Sample 117 - White-tailed Deer
(*Odocoileus virginianus*)

Bone awl



Royal Ontario Museum

Sample 960.236.224

Bone hair pin

5.4. Results Summary

The morphological preservation scale (Table 2) utilized throughout this research project was a useful tool in assessing the types of bone material best suited to this kind of sampling technique. As the drilling progressed through the four classes of material, generally the bone material became softer, more porous and easier to drill. However, it also increased the chances of making a mistake while drilling or affecting the stability of the object, both of which could result in damaging the sample more than was intended. The bone material also got harder to decontaminate effectively as it became more porous. As a result, the best material to work with for this technique is Class I and II material. However, as can be seen in the results below this technique can be used effectively on all classes of material.

The morphological analysis was undertaken to confirm that the majority of the samples were land mammals and fit the selections criteria. In addition, the comparison between the strengths and limitations of morphological based species identification and DNA based identification highlighted some interesting information. Referring to the morphological identification summary in Table 14 it is evident that the fragmentary nature of archaeological remains poses a major limitation to being able to morphologically identify the remains. An average of 54% of the selected samples could not be identified morphologically. Of those samples that could be identified morphologically the accuracy of those identifications as compared to the DNA species identification ranged from 33% at the lowest to 67% at the highest. This highlights the considerable advantage that DNA based species identification has over morphological based identification; both in its ability to identify samples regardless of their level of fragmentation, and the accuracy of the species identifications.

Table 14: Summary of morphological analysis which includes the total number of samples where a morphological identification was attempted, the number of those samples which proved too fragmentary or modified to identify, and the accuracy of the morphological identification of the samples which has corresponding DNA based identifications.

	Total Samples	Samples too Fragmented/Modified to Identify	Samples Correctly Identified
Modern Degraded	30	15 (50%)	4 of 6 (67%)
Archaeological – Fort D’Epinette	16	9 (56%)	4 of 6 (67%)
SFU Museum of Archaeology and Ethnology	6	3 (50%)	1 of 3 (33%)
Royal Ontario Museum	9	6 (67%)	0 of 0

The success rates for the drilling technique itself rose with every successive round of testing until the final Shang Dynasty Chinese artifacts from the ROM, as seen in the summary in Table 15 which indicates a reliable technique. The drop in success rate for the ROM artifacts may be attributed to the age of the artifacts (although some of the artifacts from the SFU Museum are comparable in age), or a product of the difference in climate/geography (all the testing material except the Chinese material is from British Columbia, Canada), or is the product of DNA degradation as a result of their storage in the ROM for close to 100 years. Isolating the specific factor is outside the purview of this research project, however it is likely that the Shang Dynasty Chinese material simply has less surviving DNA which decreased the success rate for that round of testing.

Table 15: Summary of the DNA based species identification success rates for each round of testing.

	Total Samples Extracted	Successful Species Identification	Success Rate
Modern Degraded	21	17	81%
Archaeological – Fort D’Epinette	16	14	88%
SFU Museum of Archaeology and Ethnology	6	6	100%
Royal Ontario Museum	9	1	11%

Additional successes include obtaining reliable sequences from bone powder amounts as low as 3mg. This is a significant result as it means it is possible to obtain the desired information with very minimal impact to the artifact. The use of x-ray imaging was also a success, both in terms of assessing any unintended damage to the samples and as a way to visually show museum professionals the minimal impact of this technique. Overall these results indicate a reliable technique which takes into account the specific needs and concerns of museum professionals and utilizes multiple tools such as x-ray imaging to address these concerns.

Chapter 6.

Discussion

6.1. Minimally Invasive Drilling Method

6.1.1. Morphological Preservation Scale

Systematically testing a range of bone material from different preservation levels, and different ages and contexts was critical to the success of this research project. By testing material from Classes I through IV it was possible to assess the strengths and challenges of this technique and determine which material is best suited for this type of minimally invasive drilling technique. The use of this standard would also make these results comparable to other similar studies using the same or similar criteria. Due to the increased softness and porosity of the more degraded bone samples (Classes III and IV), it is suggested that the better candidates for this technique are Classes I and II artifacts. These classes tend to have a more compact bone matrix, greater tensile strength, and are less fragile (see Table 2) which means they have a greater chance of remaining stable during and after the drilling process concludes. Although it has been proven possible to obtain strong, reliable DNA sequences from all four classes of bone material, the best recommendation for researchers and museum professionals is to conduct this technique on the best preserved samples available.

The bone material used in this study ranged from modern degraded bone to archaeological bone remains thousands of years old. This technique's success in obtaining DNA from archaeological remains and artifacts of this age suggest that it could be successfully applied to museum collection material from a range of contexts. However, as discussed in previous chapters there are environmental factors within the museum that may affect the probability of obtaining successful DNA extractions. There

are studies to suggest that DNA preservation is correlated with morphological preservation, specifically with collagen preservation (Campos *et al.* 2012; Götherström *et al.* 2002; Haynes *et al.* 2002; Poinar *et al.* 1996; Schwarz *et al.* 2009; Smith *et al.* 2003). Although this research project did not include any testing to determine collagen preservation, the use of a non-destructive collagen test (Vincke *et al.* 2014) could be valuable in determining which museum artifacts might be good candidates for DNA analysis, thus avoiding any unnecessary damage to museum collection artifacts.

6.1.2. Species Identification and Morphological Analysis

The morphological analysis of the testing material for this project confirmed that the vast majority of the selected bones were from land mammals. The decision to restrict the majority of the testing material to land mammals was made to limit the number of variables present in the rounds of testing so that the observations made while drilling would be more consistent.

The morphological analysis also indicated that an average of 54% of the samples were too modified or fragmented with too few distinguishing characteristics to speciate accurately. Of those samples that had enough distinguishing characteristics to speciate the success rate for the fragmented, unworked modern degraded and Fort D'Épinette samples were both 67%. In the round of testing that examined the SFU Museum artifacts the success rate fell to 33% (although the sample size was also smaller), which is less than half the success rate of the previous rounds of testing. For the ROM samples there was no overlap between the samples that were morphologically identified (only 3 of the 9 samples) and the one sample that produced a positive DNA species identification, so the success rate was zero.

One major difference between these rounds of testing that could account for the decrease in accuracy is the introduction of human modifications made to the bone artifacts. The modern degraded bones and the Fort D'Épinette material were unmodified, whereas the SFU Museum and ROM samples were all modified in some way. These modifications include carving, incising, sanding and hammering which can alter or obscure diagnostic morphological features necessary to accurately speciate the bone. In

a discipline where so much of what is studied is modified by humans it is important to have analytical techniques that are reliable, and for modified or fragmented museum artifacts the only option for obtaining this information might be aDNA analysis. As a result of the necessity of aDNA analysis to obtain certain kinds of information it is important for museum professionals to have access to sampling techniques that take into consideration their unique concerns, which may result in less hesitation when considering destructive or impactful techniques.

6.1.3. X-Ray Imaging

X-ray imaging was used during the sample preparation phase to assess the internal structure of the samples and select an optimal drilling location in order to avoid any unnecessary damage, as well as after the drilling occurred in order to assess the damage. The comparison of these x-rays side by side as well as a visual analysis indicated no substantial damage or destabilization took place as a result of the drilling procedure. As the samples and artifacts appear on the x-rays and to the naked eye to be equally as stable as they were before the drilling took place it is unlikely that this technique has affected the object's long term preservation.

One variable that was not explored during this research was the effect the x-ray imaging would have on the DNA. There are studies that assert that x-rays further damage and degrade the surviving DNA within archaeological remains (Götherström *et al.* 1995; Grieshaber *et al.* 2008; Knapp 2013). Using internal imaging such as x-ray imaging is an integral part of this technique and was deemed worth the risk of possibly degrading the DNA within the test samples and artifacts. It was outside the scope of this project to try to quantify what this affect might have been; however, this would be an excellent area for expansion in further studies. Quantitative PCR could be used to determine what affect multiple exposures to x-ray imaging might have on some samples when other variables are accounted for. Other studies have shown it is still possible to obtain short fragments of aDNA from samples repeatedly exposed to x-ray imaging and so it is expected that their effect on the samples used in this study was minimal, and still resulted in successful species identifications.

6.1.4. Advantages of a Minimally Impactful Sampling Technique

Although drilling techniques in general are not by any means a new addition to DNA sampling techniques (Barnes *et al.* 2007; Cobb 2002; Fu *et al.* 2015; Greenwood *et al.* 1999; Pichler *et al.* 2001; Rohland and Hofreiter 2007a; Shiroma *et al.* 2004), this is the first methodology that has been specifically developed with museum professional's concerns in mind and tailored for use on collection artifacts. This minimally impactful technique proceeds through every step of the DNA process, from sample selection to post-analysis long term care, with an eye to minimize areas of concern (identified in the interviews conducted as part of this research) and maximize confidence and comfort with this technique for museum professionals.

The morphological preservation scale used in the selection process was not only a useful tool in the design of this research project, but will also be useful for museum professionals when assessing collections and gauging their potential for research. Selecting appropriate samples with a reasonable chance of success at the very beginning is a critical component of this technique. There is little purpose in damaging an artifact if it can be determined through scientific assessment (ie. collagen analysis) or through museum records (ie. conservation treatment records) that the artifact is unlikely to have surviving DNA.

The drill site selection is also meant to be a joint process between researcher and museum professionals. With open communication it should be possible to select a technically sufficient location to drill while still addressing the visual and structural integrity of the artifact. The x-ray imaging built into this technique adequately assesses these concerns and informs the decisions made regarding each artifact. The imaging already completed for this research project also doubles as a way to visually show museum professionals the minimal impact of this technique. This visual evidence will hopefully bolster confidence in this sampling method and will hopefully lead to an increase in access to collections.

The technique itself has proved to be effective with a very small amount of bone powder (as little as 3 mg) and efficient with very little impact to the artifacts themselves. One issue that was raised in the museum interviews conducted for this project that was

not addressed outright in the methodology was that of a lack of knowledge or trust from museum professionals towards researchers and the techniques being used on the requested artifacts. Dr. Winter and Mr. Keddie both pointed out in their interviews that curators and collection managers may now *know* what will be done to their collections once they are loaned out for research, and this may make them hesitant to approve of destructive testing. Dr. Winter also recounted an experience where her museum loaned samples out for non-destructive analysis only to have the entire collection completely destroyed, which made her less trusting of researchers. A possible advantage to this technique is that it is theoretically portable. Given adequate space and the ability to decontaminate effectively it may be possible to drill the artifacts at the museum, under the watchful eye of a concerned curator and take the bone powder sample to be analyzed without removing the artifact from the museum. This might be an appealing compromise that increases researchers' access to museum collections while decreasing museum curators' concern over the care of their collections.

This technique is the first systematic aDNA testing method conducted specifically for use on museum collections. In addition to being a reliable, effective technique it was also designed to assuage as many fears and concerns as possible throughout the process. As such, there are many advantages and benefits to this technique specifically tailored for museum use not merely limited to the minimal physical impact to the artifacts. This thesis contains a list of DNA research recommended best practices tailored specifically for museum professionals looking to collaborate and undergo aDNA research⁶.

6.1.5. Outstanding Challenges

Although this technique has been a success there remain some outstanding technical concerns and challenges. The first and largest of which is the issue of contamination. Of the 18 modern degraded samples that yielded sequences one of them turned out to be unspecific bacterial contamination (Table 9). Of the archaeological samples, two out of the 16 Fort D'EpINETTE samples (Table 10) and seven out of the nine

⁶ See Appendix D.

Anyang samples (Table 12) yielded human contaminated amplifications. The issue here is twofold: effectively decontaminating the surface of the artifact is a challenge as physically removing the surface is a far more impactful decontamination step as it involves more damage to the artifact, and the use of universal primers as used in this study (see Table 8) will amplify everything including bacterial contamination.

The largest challenge with this study, and in large part the reason for the multiple rounds of testing, is how to decontaminate the samples effectively. Regular decontamination protocols are more destructive and visually noticeable as they include the use of plenty of bleach and the physical removal of a portion of the contaminated surface in order to access the interior of the bone. It is assumed that the interior of the bone is less contaminated, but this is highly debatable and would depend on the type of bone (compact vs. trabecular) and the depositional conditions. These methods were deemed too impactful for the focus of this technique and so a less extreme measure was used. The method used in this technique is less impactful to the artifact however any residual contamination can easily overwhelm the PCR process. This problem is compounded by the use of very general universal primers which will capture and amplify everything remaining after the decontamination process.

More specific primers would help exclude some contaminants, and should be used when possible. Another solution to this problem might be pairing this technique with next generation sequencing. With this method everything extracted from the sample is sequenced, including contaminant DNA, and those sequences are removed post-extraction in the subsequent bioinformatic analysis. What is left after this cleanup process is the authentic artifact DNA regardless of the thoroughness of the decontamination protocol. As a result, many labs that employ next generation sequencing do not attempt to decontaminate at all as contaminants can easily be removed post-sequencing and the decontamination process may destroy the desired authentic aDNA. Although next generation sequencing is becoming less expensive over time it still costs approximately \$1000 a sample, which can be prohibitive for many researchers.

The other outstanding challenge is the success rate for the fragmentary Shang Dynasty Anyang artifacts from the ROM. In the first round of testing seven of the nine artifacts were contaminated by human DNA (Table 12). These artifacts have been in the ROM's collections for almost 100 years and have been handled by many museum professionals during that time. The decontamination protocol was clearly not as effective on these samples as it was on the previous three rounds of testing. In addition, the authentic DNA in these artifacts appears to be highly degraded. This degradation may be the result of age, however the McNaughton Island, Noons Creek and Crescent Beach artifacts from British Columbia are of comparable dates and still produced a reliable species identification. The highly fragmented state of this DNA may also be a product of the difference in climate/geography, the possibility that they were heated in ancient times which would rapidly degrade the DNA (the oracle bones would have been heat treated), or as a result of their storage and handling (they were rehoused within the last five years and are largely stored on open air trays) in the ROM for close to 100 years.

Another possibility, which would require further research to confirm, is that the speed of the drill may have contributed to a further decline in the already fragile and fragmented Anyang DNA. Other labs that utilize drilling techniques suggest low drilling speeds around 100 rpm so that the drill does not create any additional friction and heat that could further degrade the DNA (Barnes *et al.* 2007; Greenwood *et al.* 1999; Pichler *et al.* 2001). The drilling for these samples was done at 1,000 rpm (the lowest setting on this drill model) and may have contributed to a further decline in DNA due to the fragility of these samples. If possible, it may be wise to invest in a drill that starts at a lower rpm range in order to have more versatility in drill speed options. Any type of drill could be used as long as it has a versatile speed range (100 - 2,000 rpm) that can be tailored to the material one is working with. In practice, the drill model itself is less important than the drill bits used for the sampling.

6.2. Implications for Museums and Museum Collections

This research project aimed at developing a reliable minimally destructive technique for extracting bone samples for aDNA analysis and was designed based on the assumption that the destructive nature of aDNA sampling acts as a barrier to

accessing museum collections. This assumption is supported in the literature (Adriaens 2005; Baker 1994; Bolnick *et al.* 2012; Lehmann *et al.* 2005; Mundy *et al.* 1997; Rohland *et al.* 2004; Thomsen *et al.* 2009; Wisely *et al.* 2003) as well as the interviews conducted for this research project with museum professionals from three regions of Canada. Of the ten museum professionals interviewed, 80% identified destruction/damage to the artifact as the main barrier to DNA testing on museum collections. However, none of the interview participants were against destructive testing in principle. Rather, many of them felt museums have an important role to play in contributing to the world's knowledge and wealth of information but a balance would need to be struck between conservation and research potential.

To this end there are a number of non-destructive and minimally destructive techniques available that balance the factors that are important to museum professionals. As summarized in previous chapters a frequently used non-destructive technique involves the submersion of the artifact, or part of an artifact, in a digestive solution (Bolnick *et al.* 2012; Gilbert *et al.* 2007; Phillips and Simon 1995; Rohland *et al.* 2004). This method is visually non-destructive in the short term; however there have been issues with consistency among the samples. Not all the objects subjected to this technique have reacted equally. Some objects have been the subject of excess tissue loss during the submersion process. This inconsistent reaction, or the possibility of unknown longer term damage over subsequent decades after testing, may make museum curators, collection managers and directors that are most concerned with long term preservation hesitant to allow this form of testing. Alternatively this could be the most appealing technique to those museum professionals that are most concerned with the intact aesthetic appearance of the object (for example, if it is selected to be displayed in an exhibit).

Alternatively, there are many minimally destructive techniques that focus on sampling in unobtrusive locations (Horváth *et al.* 2005; Wisely *et al.* 2004), or minimizing the impact to the external appearance of the sample (Shiroma *et al.* 2004; Wisely *et al.* 2004). These techniques may be most appealing to those museum professionals that are concerned with long term preservation and stability, or those that wish to keep the sample as chemically unaltered as possible so it can be re-examined using other forms

of analysis (for example, carbon 14 dating). These forms of analysis are destructive to some portion of the external surface of the object, and therefore some museum professionals may feel this technique is inappropriate for use on their collections. The ability to easily fill any holes or scoring with wax or polymer so that the object appears untouched may assuage some concerns from museum professionals.

The technique presented in this thesis provides a technique for minimally impactful DNA sampling that was specifically designed with the concerns of museum professionals in mind. In order to address the concerns regarding the physical damage to the artifact, the largest hole that would be drilled is less than 2 mm in diameter and could be drilled in an unobtrusive location. Even without filling the hole it would still be possible to display any of the artifacts post-drilling. This technique also addresses the issue of long term preservation. No digestive buffers are used on the artifact which could lead to degradation or destabilization over time. In addition, the use of x-ray imaging pre and post-drilling ensures that the artifacts are stable throughout the process. The side by side x-ray comparison in this study shows no significant or unnecessary damage is done to the artifacts, and that they are just as stable as when the sample selection process began. As a result, no long term damage or instability is expected to occur as a result of this technique. The artifacts can be returned to their collections without fear of further degradation or destabilization caused by the DNA sampling protocols.

6.3. Future Studies

This research project was built upon three decades of cumulative knowledge and exploration, and touches upon many important areas of research that were unable to be addressed within the bounds of this project. These are some areas of research that are directly related to this study and are deserving of further consideration and analysis.

6.3.1. DNA Extraction Techniques and Long Term Stability

When looking for the least destructive or impactful method to use on archaeological remains or artifacts there are many options available. Many of these minimally destructive and non-destructive techniques have been developed and

published within the last 15 years. Some of these publications have detailed their own limitations, a common one being the lack of long term studies to detail the effects of these techniques over time. A systematic comparison of these techniques combined with a long term component showing the effects of these techniques over time would be of tremendous benefit to researchers and museum professionals. A study detailing the long term strengths and drawbacks of non-destructive chemical submersion techniques versus minimally invasive drilling techniques would be able to lend hard facts to the possibilities only theorized in this thesis.

6.3.2. Further Decontamination Options

As seen with the Shang Dynasty Anyang artifacts there is still room for improvement with the decontamination protocol. All the bleach used in this research project was 100% (5% sodium hypochlorite) bleach, including the experiment that tested the effects of directly bleaching the bone powder (Figure 5). It may be possible to directly bleach the bone powder without losing the authentic DNA sequences if the bleaching was done at a lower concentration. The use of UV light for decontamination was also not tested in this project, but may end up providing a viable option which could increase success rates.

6.3.3. DNA Degradation in Museum Artifacts

The possibility that some museum artifacts may not make successful candidates for DNA analysis has been addressed in earlier chapters. Many of the museum professionals that agreed to be interviewed for this research project spoke about a balance needing to be struck between the need for research and the ethical responsibility they have to preserve and care for museum collections. One of the core statements that came from these individuals stressed the need for the damage to the artifact to be “worth it”. In order for the DNA analysis to have the best chance possible of providing constructive, worthwhile information care must be put into selecting artifacts that have the best chance for success.

In order to achieve this researchers and museum professionals must work together to target artifacts that both appropriately address the research questions, while taking into consideration their storage and treatment conditions.

As secondary storage environment, museums environments can be responsible for further DNA damage and degradation (Burger *et al.* 1999; Campos *et al.* 2012; Phillips and Simon 1995; Wandeler *et al.* 2003; Willerslev and Cooper 2005) and so it is important to select artifacts for analysis that have been stored in conditions that avoid humidity, greater access to oxygenation (example, on a shelf in a high traffic area) or water (washing), heat or temperature fluctuations, and acidic storage paper or boxes. Selecting appropriate artifacts at this stage will maximize the chances of success. A challenge to this however is the nature of museum record keeping in older museums as it is rarely possible to know under what conditions an artifact was stored at every point in its life in the collection. In addition, there might not be any information regarding how they were cared for or stored before the artifact came to the museum.

There are many studies that have detailed the effects of these conditions on DNA degradation in depositional environments (Burger *et al.* 1999; Campos *et al.* 2012; Phillips and Simon 1995; Smith *et al.* 2003; Smith *et al.* 2003; Wandeler *et al.* 2003; Willerslev and Cooper 2005), however there have been fewer studies detailing their effects on artifacts in a museum environment (Rohland *et al.* 2004; Wandeler *et al.* 2003). More systematic studies aimed at isolating these various factors within a museum environment would help with improving bone storage conditions within museums and would greatly increase chances of success for DNA analysis.

6.3.4. Museum Conservation and Inhibition

In addition to storage conditions, conservation work may also have an effect on chances of success for DNA analysis. Conservation treatments have changed dramatically over time as have the materials used in these treatments. It is possible that bone artifacts that have been treated with glue, nail polish, hair spray, etc. may not be ideal candidates for DNA analysis as the consolidants and chemicals found in these substances may act as inhibitors during the PCR process.

Research into the effects of common modern conservation treatment materials, such as cyclododecane, polyethylene glycol, or polyvinyl acetate glues would be incredibly important to DNA researchers and museum professionals. A guide on what treatments may create inhibition would be of critical importance during the sample selection phase. Museum professionals and researchers could compare this list with museum conservation and curation documents and make informed decisions before any destructive testing even takes place.

Chapter 7.

Conclusion

The research project has examined two critical perspectives regarding the use and care of archaeological collections and brought them together to develop a successful, comprehensive and reliable minimally impactful DNA sampling technique that is tailor-made to address the concerns and ethical responsibilities of museum professionals. This study is the first of its kind to directly identify the concerns of museum professionals by conducting a series of interviews, and tailoring this sampling technique to address the specific concerns of the museum professionals that control access to archaeological collections in Canada.

The interviews conducted with an array of museum professionals from three regions of Canada indicated that none of the participants were against destructive testing in principle but rather, a balance would need to be struck between conservation and research potential. The interview participants felt that museums have an important role to play in contributing to the world's knowledge wealth of information but a lack of transparency and equal collaboration from researchers, and the destruction necessary to obtain DNA sequences from an artifact make museum professionals hesitant to allow destructive testing on their collections. As a response to these concerns this technique offers a range of assurances that should assuage their fears and hopefully grant greater access to these collections for research.

This minimally destructive technique uses a micro drill that drills into the external surface of an artifact with an opening a maximum of 2mm in diameter to extract a small amount of bone powder (from 3-40 mg) for testing. As the DNA extraction is being conducted on the resulting bone powder there is no further impact upon the artifact such

as digestive chemicals which could affect the long term stability of the object in subsequent years after the artifacts are returned to the museum collections.

In addition to the physically minimally impactful nature of the technique, the morphological preservation scale and x-ray imaging built into the methodology of this technique make it possible to assess each artifact's viability before physically impacting the object. Within this project the morphological preservation scale served as a guideline for systematically testing a range of material preservation levels to identify the optimal material and identify the strengths and weaknesses of the technique. Use of this morphological preservation scale by museum professionals and researchers will also allow them to discuss with shared, consistent terminology which materials from their collections would be best suited for the proposed research.

X-ray imaging is also a critical tool within this technique as it allows those involved to assess the internal structural integrity of the artifacts before any drilling is conducted and select a precise drilling location. This precision will allow the researcher to avoid instable areas or rule out the artifact entirely if drilling into the object is likely to create more damage than intended. The comparison of the pre and post-drilling x-ray imaging in this research project also provides a reference for museum professionals which can visually confirm that the drilling has not caused any additional unintended damage to the artifacts, which will hopefully go a long way to assuaging any fears regarding the impact of this technique.

The drilling technique itself has proved to be successful with an overall success rate of 73% in species identifications throughout four rounds of testing, using small amounts of bone powder and universal primers. An outstanding challenge identified in this research that can be further optimized is the decontamination protocol which relied on a bleach treatment. The bleach was applied to a localized area on each artifact in order to reduce discolouration and possible long term damage or further DNA degradation over time. Further optimization of this decontamination protocol, or utilization of next generation sequencing to remove contaminant DNA post-extraction would further increase the success rate of this technique.

This research project has demonstrated the success of this technique on ancient, valuable museum artifacts and has included a number of safeguards specifically designed to respond to the concerns of museum professionals concerned for the integrity of their collections. It is hoped that this technique will instill greater confidence in DNA extraction methods and allow for greater access to museum collections in the future, which could add untold insight into the world.

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Appendix A.

Consent Form

Developing Minimally Destructive Protocols for DNA Analysis of Bone Artifacts

Version #3 2016s0040 – February 4, 2016

Principal Investigator:

Kelly E Brown, MA Student, Department of Archaeology

Senior Supervisor:

Dr. Dongya Yang, Professor, Department of Archaeology

SFU Collaborator:

Dr. Barbara Winter, Director of the Museum of Archaeology and Ethnology

Sponsor:

This research is funded, in part, by the Social Sciences and Humanities Research Council (SSHRC) and by Simon Fraser University.

This research study is being undertaken as part of a Master of Arts degree. The results of this study will be part of a thesis, which will be public knowledge and located online and in the Simon Fraser University Library collections.

Simon Fraser University and Kelly Brown, the researcher conducting this study, subscribe to the ethical conduct of research and to the protection at all times of the interests, comfort, and safety of participants. The chief concern of the Board is for the health, safety, and psychological well being of research participants.

Invitation and Study Purpose:

You are being invited to take part in this study because you are a museum professional in charge of the conservation and preservation of the collections under your care. By virtue of being a curator or collections manager you are in a position within your institution to make decisions regarding research access to those collections.

My research is focused on developing a minimally destructive sampling protocol for extracting ancient DNA (aDNA) from bone artifacts curated in museum collections. My goal in conducting this research is to offer a less destructive way of sampling bone artifacts which may result in increased access to previously inaccessible museum collections. Through this interview I hope to learn about your thoughts, experiences and concerns regarding research on the archaeological collections in your care.

Voluntary Participation and Withdrawal:

Your participation is voluntary. You have the right to refuse to participate in this study. If you decide to participate, you may still choose to withdraw from the study at any

time without giving a reason. If you choose to withdraw from the study, I will immediately destroy all data collected from or about you during your participation.

As this study does not include the collection of any secondary data (database records, government records etc.) or include access to any of your institution's records (collocation management records, conservation reports etc.) I will not be obtaining permission from your institution or organization.

Study Procedures:

If you agree to participate, this is how the study will proceed:

- I will schedule an interview time and location with you – ideally a face to face meeting, or phone or Skype interview depending on your preference and my ability to travel.
- The interview will take approximately 1 – 1 ½ hours of your time. I will ask you questions related to the study's topic, as well as invite you to freely share your thoughts, feelings and concerns in a conversational style interview.
- I feel audio recording will be necessary in order to capture the whole, nuanced conversation, as furious note-taking might distract from the flow of the conversation. I acknowledge that some people may find this uncomfortable and so I hope that you will consider allowing me to record your interview, but I leave the choice up to you:

Yes, I **agree** to have this interview audio-recorded.

If you consent this interview will be audio recorded, and then transcribed within two weeks. The audio recording will be immediately destroyed after transcription. All data will be kept in a lockbox located within a filing cabinet in my locked office, and will only be accessed by myself.

Potential Risks of the Study:

This study is designated “minimal risk” and there are no foreseeable risks to you participating in this study.

Potential Benefits of the Study:

I do not expect that there will be any direct benefits to you from participating in this study. However my hope is that by contributing to this study you will be helping researchers understand the concerns of museum professionals. This understanding will enable researchers to develop more appropriate research techniques which may render previously inaccessible material available for research and allow us to construct more meaningful interpretations of the past. During the course of your interview you may also find some of the technical information I present to be interesting or useful in your professional decisions.

Confidentiality:

If you request that your identity remain confidential, I will maintain confidentiality of your name and any identifying information within all documents produced that are related to this study, to the extent allowed by the law. If you choose to have your information anonymized it then may not be possible to remove you from the study if you choose to withdraw after your interview as your information will be unidentifiable. Please note that confidentiality cannot be guaranteed if interviews are conducted over the

phone, email, or Skype, as they are unsecure mediums. Please choose one of the following:

- I would like my identifying information to remain confidential.
- I consent to identifying information being used in this study.

Please let me know if there is any additional information that cannot be made public.

Study Results:

You may obtain copies of the results of this study upon its completion by contacting Kelly Brown; or Dr. Dongya Yang; or Dr. Barbara Winter.

The results of this study will be reported in a graduate thesis and may also be published in journal articles or presented at academic conferences. All “published” material produced as a result of this study will be made available to study participants. If you would like to be notified by e-mail when new material related to this study is published please check the box below. You may opt out at any time.

- Yes, please keep me notified when new material related to this study is published.

Remuneration:

No remuneration is offered for those electing to participate in this study.

Contact for Complaints:

If you have any concerns about your rights as a research participant and/or your experiences while participating in this study, you may contact Dr. Jeffrey Toward, Director, Office of Research Ethics.

Future Use of Participation Data:

The data you choose to provide will be used in a strictly academic context, towards the creation of my MA thesis as well as any associated academic material (conference posters, lectures, journal articles). Confidential data may be re-used in future research projects. However, any information or quotations directly associated with your interview will not be used in future research without first re-obtaining consent from you.

Future Contact:

The information you have contributed may be used in future studies:

- Yes, I **agree** to this information being used in future studies.
- No, I **do not agree** to this information being used in future studies.

Participant Consent:

Taking part in this study is entirely voluntary. You have the right to refuse to participate in this study. If you decide to take part, you may choose to pull out of the study at any time without giving a reason and without any negative impact.

Your signature below indicates that you have received a copy of this consent form for your own records.

Your signature indicates that you consent to participate in this study.

Signature: _____ Date: _____
YYYY/MM/DD

Printed Participant Name: _____

Appendix B.

Interview Questions

DNA Research Questions

Could you describe what you do in the museum and how long you have been working here?

Does your institution have artifacts in the collections made from bone?

If so, approximately how many artifacts are made wholly or in part from animal bone?

Approximately how old are your bone artifacts (date ranges)?

In your opinion what is the greatest barrier to DNA testing on museum collections?

Has your institution had any DNA research conducted on your bone artifacts?

If so, what did you learn about the artifact that you did not already know? What was the benefit to having this research conducted?

Do you think the knowledge gained justified the damage done to the artifact?

If there was a technique that would cause no damage at all, is there any DNA research that you or your institution would like to have done (research questions)?

Does your museum have a destructive testing policy or protocols?

Have you ever had destructive testing done on any of your collections?

If so, what was your reaction? Experience?

What are the key factors that would weigh in your decision to allow destructive testing on your bone artifact collections?

Would you be concerned about long term preservation, physical damage, appearance, physical stability of the object etc.?

Are there some artifacts that you would allow destructive testing on, and some artifacts that you would not?

What do you see as the future of DNA research in museums?

Collection Management Questions

When did this museum first opened and where did the first collections come from?

When was the earliest bone artifact collected and how long has it been in your collections?

Do you have treatment records detailing what possible treatments have been applied to your bone artifact collections, especially from the early days? (cleaning, consolidation techniques, adhesives, pesticides etc.)

What conditions are your bone artifact collections stored in (humidity controlled, temperature controlled, ventilation, boxes/shelves etc.)?

Have the bone artifacts always been stored in these conditions?

Does your institution currently have any official protocols for curators, collection managers and/or conservationists for the treatment, handling and/or storage of your bone artifact collections?

Appendix C.

X-Ray Imaging Comparison

The image on the left of each pairing is the pre-drilled x-ray, and the image on the right is the post-drilled x-ray.

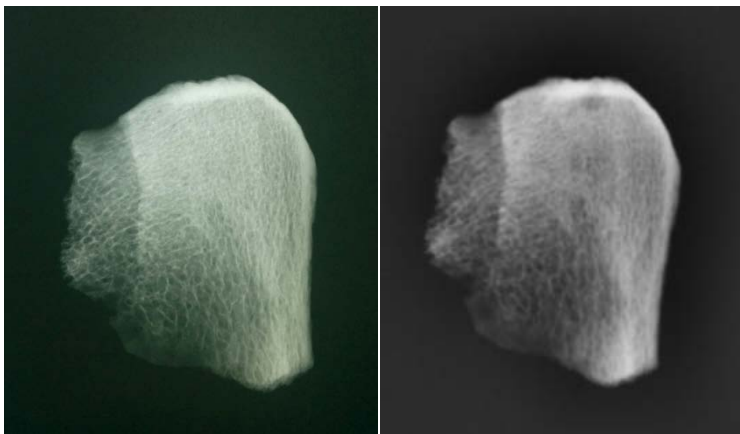
Fort D'EpINETTE Samples Material



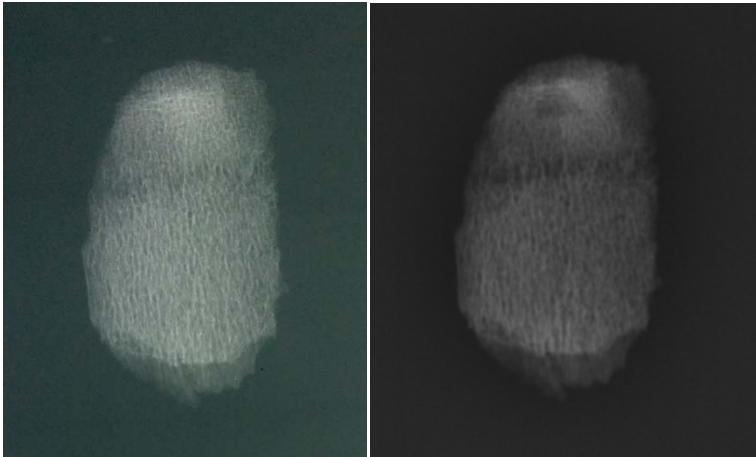
Sample 301 - Red deer/ Elk (*Cervus elaphus*)



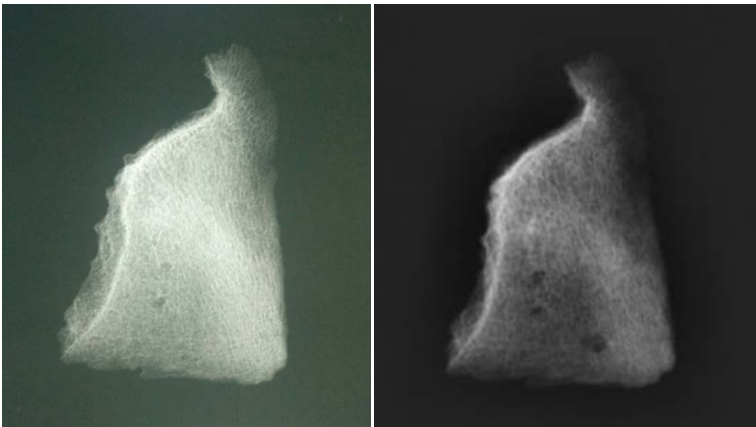
Sample 302 - Moose (*Alces alces*)



Sample 303 - Red deer/ Elk (*Cervus elaphus*)



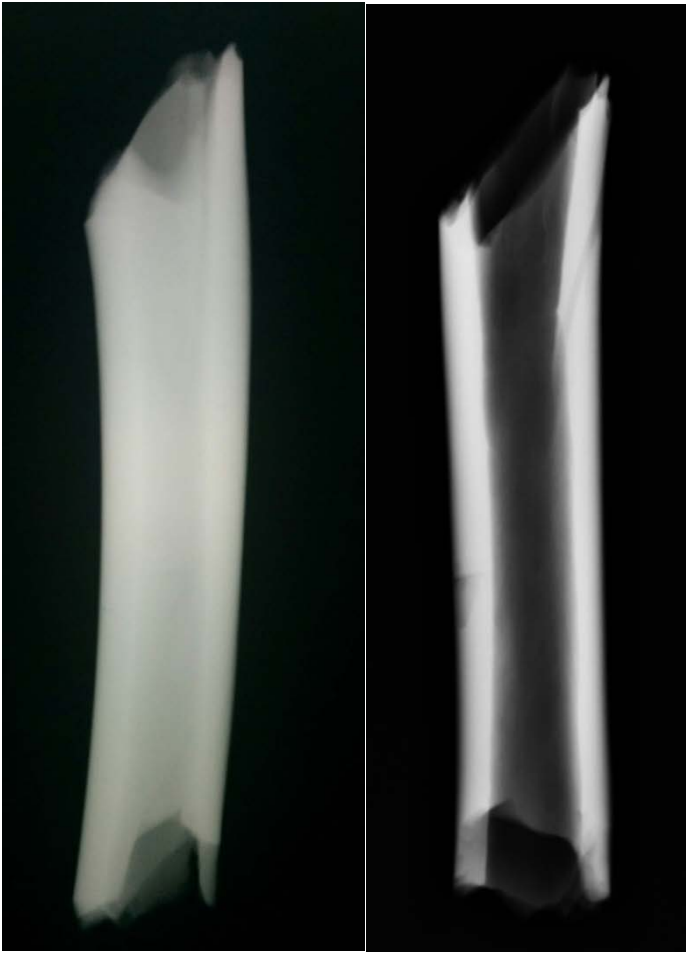
Sample 304 - Moose (*Alces alces*)



Sample 305 - Moose (*Alces alces*)



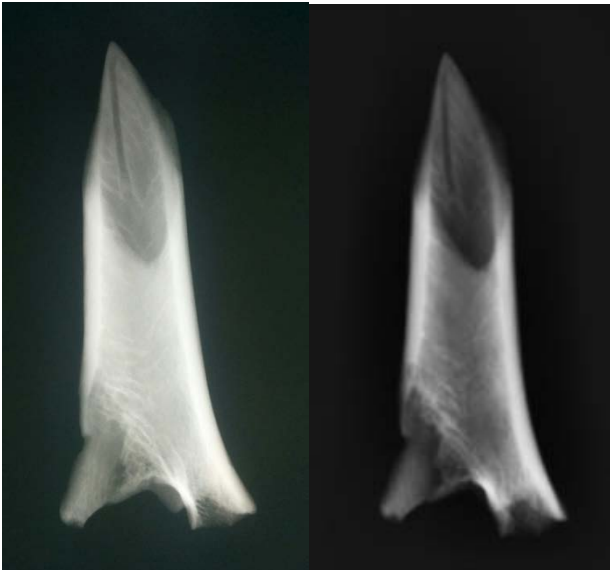
Sample 306 - Red deer/ Elk (*Cervus elaphus*)



Sample 2957 - Red deer/ Elk (*Cervus elaphus*)



Sample 3095



Sample 4057 - Gray wolf (*Canis lupus*)



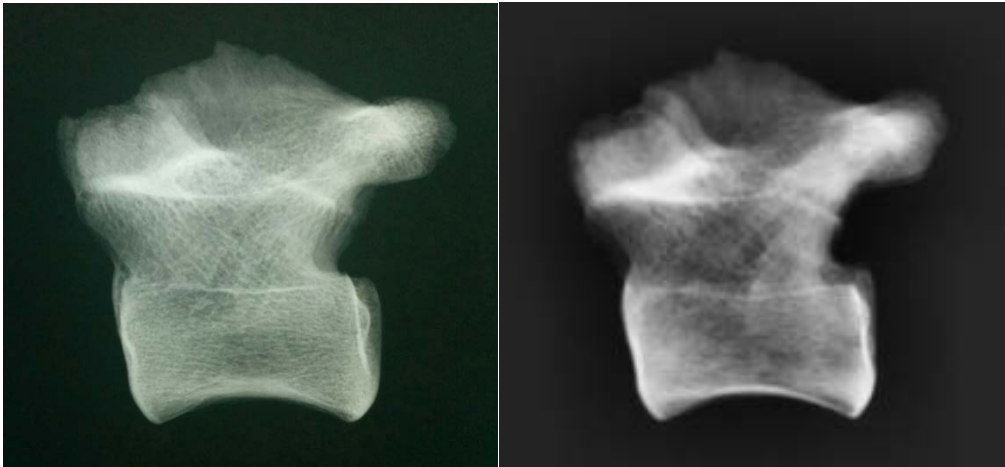
Sample 6352 - Gray wolf (*Canis lupus*)



Sample 6858 - Gray wolf (*Canis lupus*)



Sample 27:2042



Sample 27:709 - Gray wolf (*Canis lupus*)



Sample A25E1 - Moose (*Alces alces*)

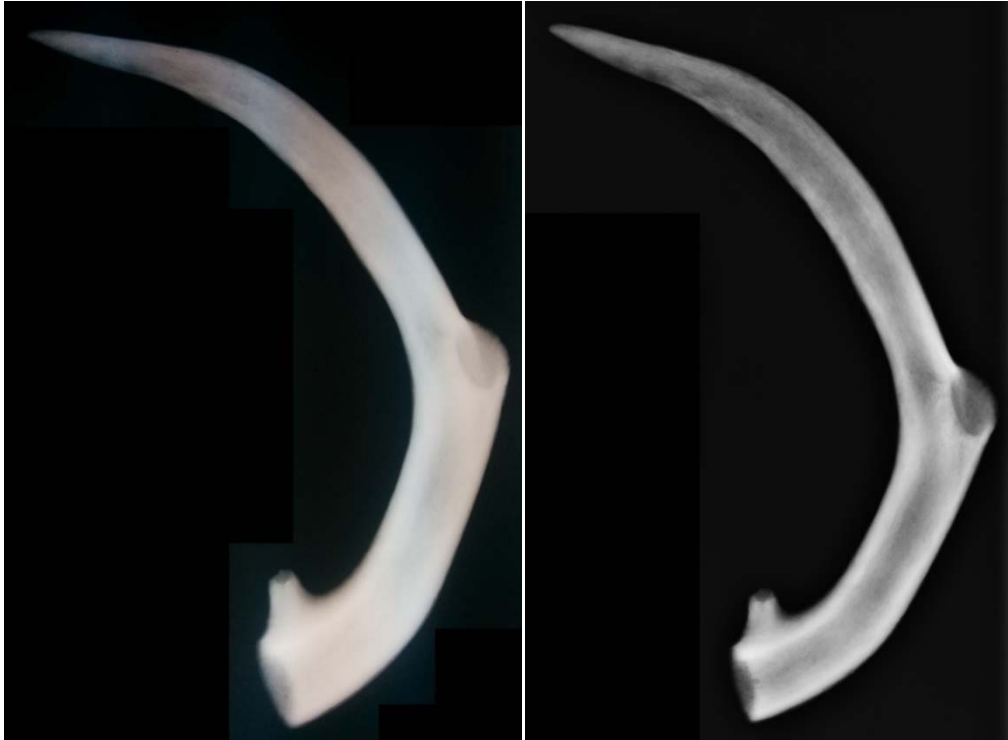


Sample A45E10 - Moose (*Alces alces*)



Sample A7-2 - Moose (*Alces alces*)

Anutcix (FaSu-10)

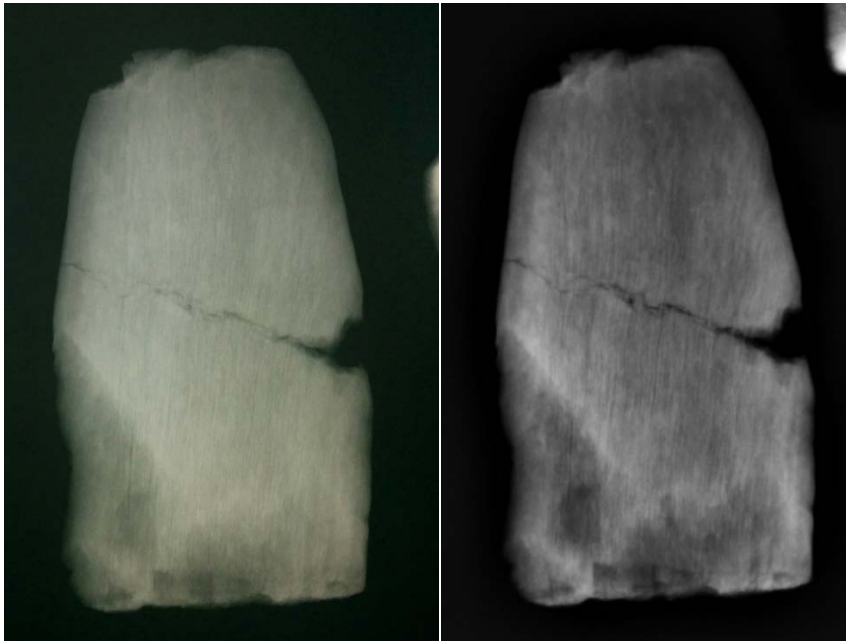


Sample 100 - White-tailed Deer (*Odocoileus virginianus*)



Sample 948 - Sea Otter (*Enhydra lutris*)

Noons Creek/ Say-mah-mit (DhRq-1)



Sample 314 - Red Deer/Elk (*Cervus elaphus*)



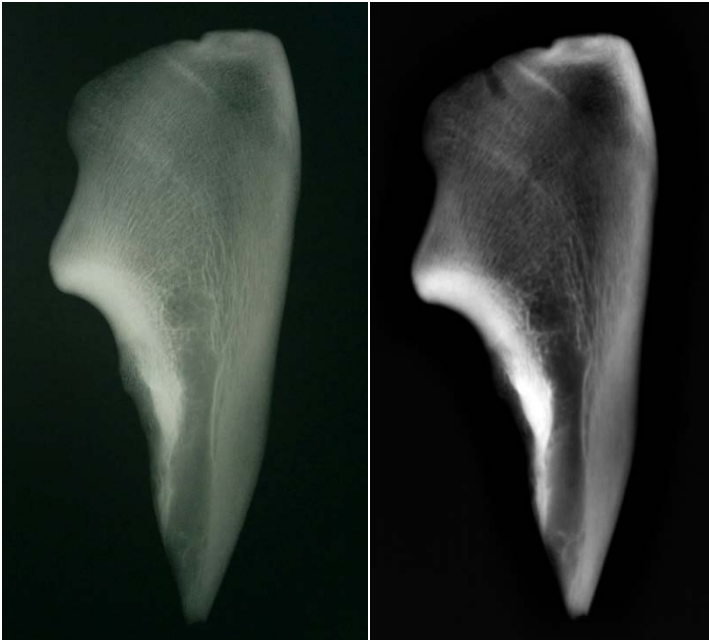
Sample 323 - Red Deer/Elk (*Cervus elaphus*)

McNaughton Island (EITb-10)



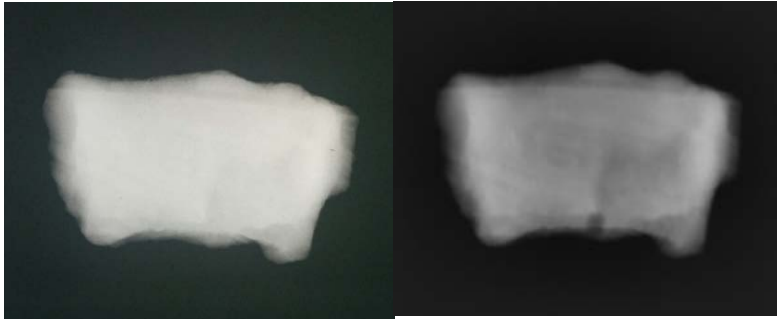
Sample 459 - White-tailed Deer (*Odocoileus virginianus*)

Crescent Beach (DgRr-1)



Sample 117 - White-tailed Deer (*Odocoileus virginianus*)

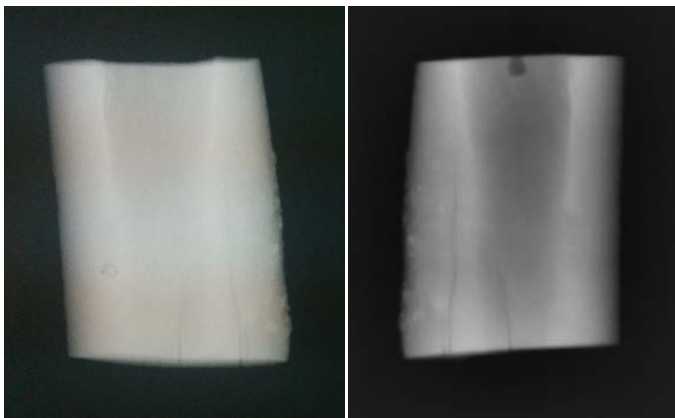
Royal Ontario Museum Artifacts



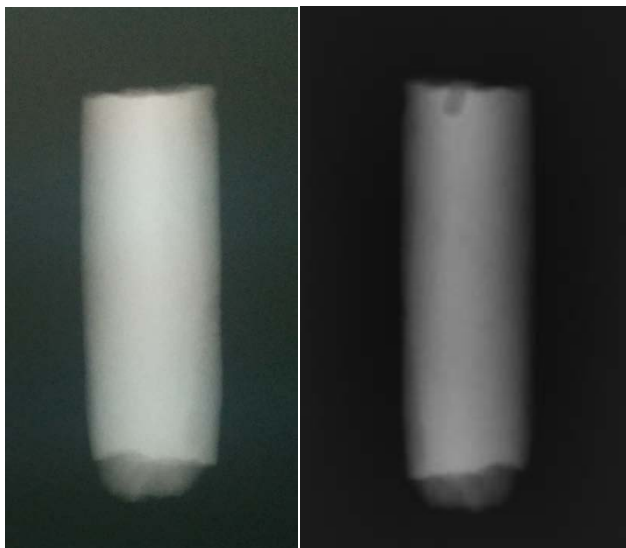
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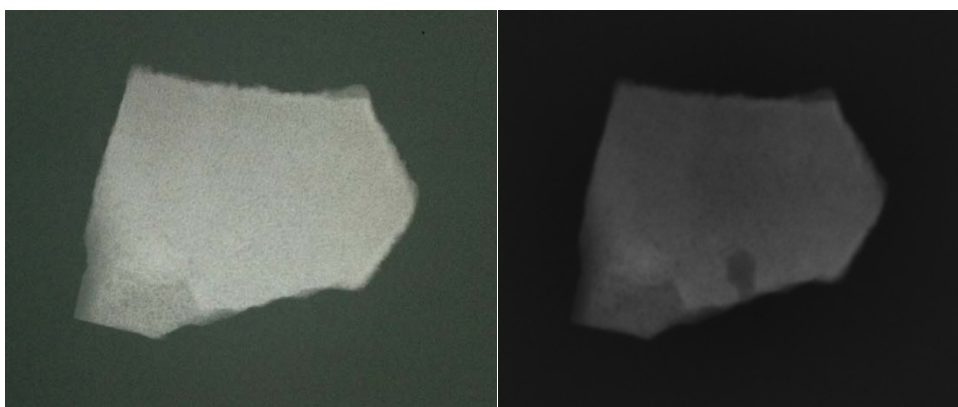
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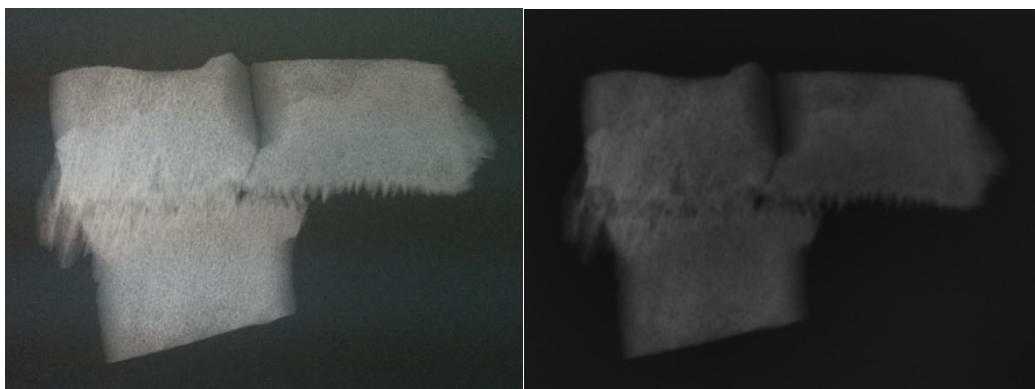
960.236.383.1 – Cow (*Bos taurus*)



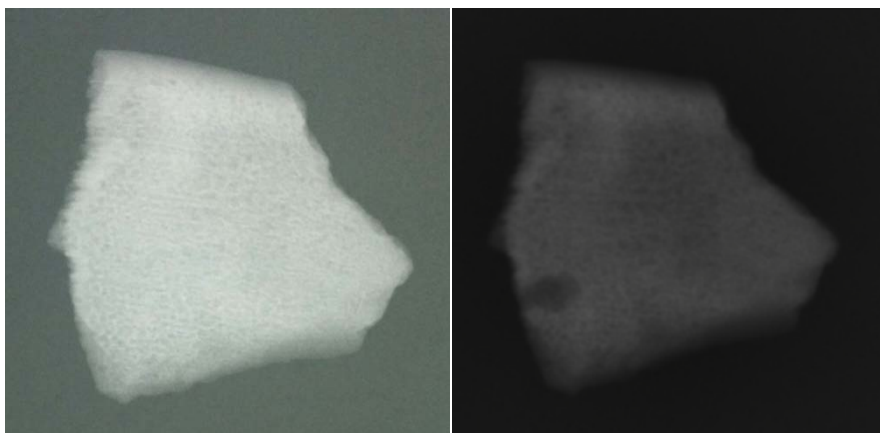
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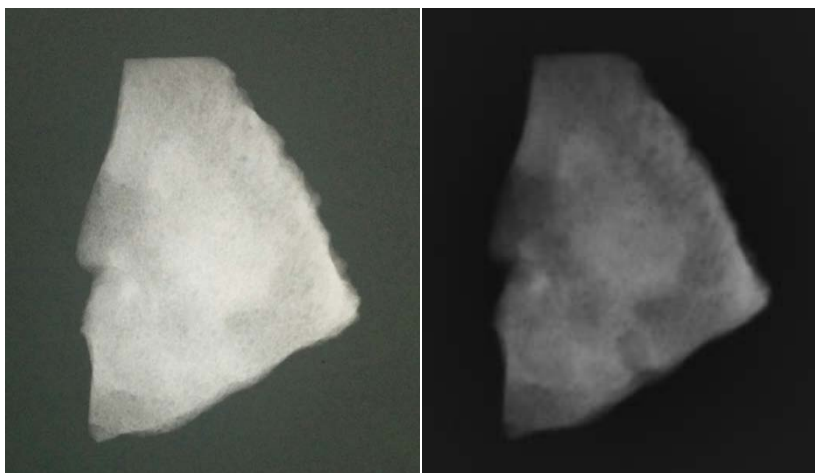
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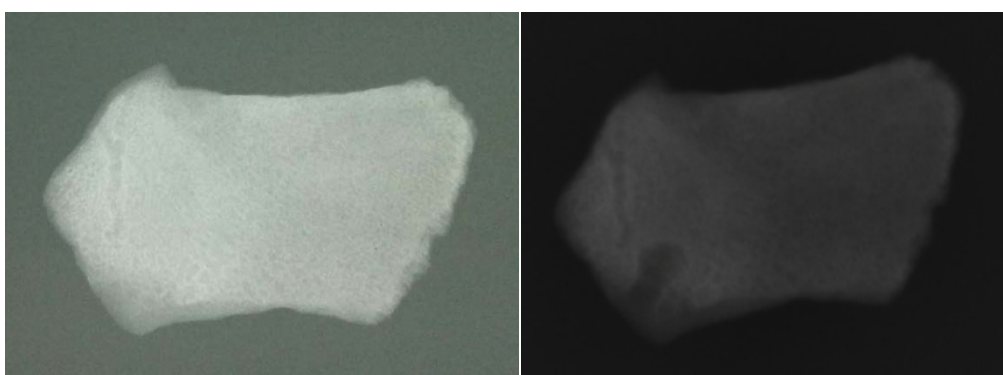
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Appendix D.

DNA Research Recommended Best Practices Guide for Museum Professionals

The following steps are a list of recommended best practices based on the results of this research project. These steps are meant to aid museum professionals in selecting museum collection artifacts with the greatest chance of providing amplifiable DNA sequences. The following steps are assuming that the museum professionals are collaborating with external researchers seeking to use collection artifacts for research. If this research is proposed and taking place in house some of these steps are redundant or unnecessary.

1. Identify your research question:

- What information are you looking to get from this research? Is this information that will be a valuable contribution to the museum, researchers, universities, and/or global knowledge?
- How many artifacts are necessary to obtain the information you are looking for? For example, do you want a species identification for a particular object (only one artifact is necessary), or are you trying to identify the favoured material used for making a class of artifact (many artifacts will need to be analyzed)?
- What is the cost associated with this research? Who will be paying for the analysis?
- How will credit/authorship be established?
- How will the product(s) of this research be disseminated? For example, is this information to be displayed in an exhibit, an online database, a free access publication, or a journal publication (which may not be freely accessible)?

2. Alternative information sources:

- Is it possible to get this information another way? For example, is it possible to identify the favoured material for making a class of artifacts using ethnographic sources? Have other researchers or museums done similar research?
- Is there associated material such as soil that could be tested instead of the artifact?

3. Assessing Preservation:

- Once you have determined that it is necessary to test collection artifacts and how many are needed to answer your research question, you can start browsing your collection for suitable artifacts to sample.
- Using the morphological preservation scale below start to classify your collection. Are there are Class V artifacts that may not be suitable for display or other kinds of research that could be wholly used for DNA analysis (rendering this technique unnecessary)?
- If not, look for bone artifacts from Classes I-III that are likely to remain stable throughout the drilling (DNA sampling) process.

Morphological Preservation Scale

This scale is an amalgamated version of similar previously published scales (Haynes et al. 2002, Petchey and Higham 2000, and Stafford et al. 1988).

Class I	Class II	Class III	Class IV	Class V
High compressive and tensile strength; spiral and conchoidal fracturing; waxy luster; dense bone matrix	Bone is chalky with loss of conchoidal fracturing; exterior still hard and waxy	Interior and exterior of bone are chalky; surface hardness decreases and porosity increases	Continued decrease in hardness and increase in porosity	Soft, easily pulverized bone if no inorganic replacement occurs, low density; hard if inorganic replacement has occurred

4. Consult museum records:

- Once the prospective samples are selected check all records associated with each artifact. There are a lot of factors within a museum environment that can increase DNA degradation, which will reduce the chances of an artifact providing successful sequences.
- *Storage and environmental conditions:* Elevated or fluctuating temperatures and humidity can rapidly degrade any surviving DNA. Greater access to oxygen or water is also a factor that can degrade DNA, as well as storage in acidic boxes, shelves or paper. Are your artifacts stored in controlled environments? Are they stored on open shelving units near a high pedestrian area (increased air flow) or in closed boxes? Have these storage conditions changed since they originally entered the collection (re-housing)?
- *Handling:* DNA degradation can be accelerated by human handling. This can also greatly increase the chances of human DNA contamination, especially if the artifacts are handled without gloves. Has your sample been consistently handled in its time in your collection?
- *Conservation work:* Any chemicals or compounds that have been used to treat the artifact may have an inhibiting effect on DNA amplification. Some of these treatments might also degrade DNA over time. Has your artifact been treated at any point, or has it remained largely untreated?
- **Summary:** When selecting artifacts for sampling look for samples that have been in stable environmental condition, stored in non-acidic boxes or shelving that have not been subject to regular air flow, and that have been handled and treated as little as possible.

5. X-Ray Imaging:

- Once appropriate artifacts have been selected for analysis you should discuss them with the researchers (if they were not part of the initial selection process), to ensure that these samples will still fit into your research question.
- If they do, proceed to x-ray imaging the samples. These images should be viewed by both yourself and the researcher to ensure that the artifacts are indeed stable for drilling.

6. Consultation with researchers:

- There should be open and equitable communication between the researchers and museum professionals.
- Using the x-ray imaging as a guide, a drill site will need to be selected. This should be a collaborative process. The researcher may have technical requirements for the drill site, and museum professionals may have conservation/display concerns.
- Is this an artifact that is going to be displayed? If so, inform the researchers and make sure they select a drill location that does not compromise the research goal or the aesthetic of the artifact. It may be possible to fill the resulting hole with resin or wax which may make the drill location less important for display purposes.

7. Return of the samples:

- The artifacts should be treated with appropriate care by the researcher when in their custody (provide them with handling instructions if necessary) and processed in a timely manner (agreed upon beforehand).
- If there are any retests necessary (additional holes to be drilled, or additional powder extracted), this should be discussed with you first.
- Once the sampling is done and no further tests are necessary the artifacts should be x-rayed again to ensure no additional damage has been done, and then returned to the museum as per your loan agreement.

Hopefully this list of recommended best practices will provide a useful outline to aid in the selection of artifacts with the greatest chance of success for DNA analysis. These steps also highlight codes of behaviour that can be expected of collaborators and questions to think about when engaging in such a research partnership.